



# **STUDY AND MODELLING OF THE CARBON CYCLE THROUGH PLANT ORGANS AND MICROORGANISMS OF A LEGUME-CEREAL AGROSYSTEM**

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## Abstract

The objective of this internship was to collect the data necessary to apply a Model of the transformations of Organic matter by Micro Organisms of the Soil (MOMOS) to one cultural cycle of a legume-cereal agrosystem in the region of Languedoc-Rousillon, France. Three modes of culture were studied: faba bean, durum wheat and the association of faba bean and durum wheat. Experimental data was collected on C and N content of microbial biomass, plant organs and soil. Soil respiration was measured on the field. The previously determined values for MOMOS optimal parameters, relationships linking parameters to quality of inputs, soil texture and climate, were preserved in this study. Within our Mediterranean context, the only changes introduced to MOMOS (which had been calibrated and validated in acidic tropical conditions) were the local correction factors of temperature and moisture, quality of plant materials and edaphic properties. The plant production module was also adapted to suit the experimental set up. Experimental data was then plotted against model predictions for MOMOS-C (carbon). Results showed proximity between measured and predicted values of carbon content in plant organs, soil and microbial biomass. Field respiration measurements which included respiration of active roots were found to be quite higher than predicted microbial heterotrophic respiration. Model predictions indicated higher microbial C content and higher microbial respiration activity in wheat parcels than in faba bean. Microbial biomass and microbial activity responded quickly to changes in environmental conditions such as soil moisture levels and inputs of plant necromass.

**Keywords:** microbial biomass, model, carbon, nitrogen, organic matter transformations, cereal-legume associations

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## Introduction

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The issue of global food security is a challenge confronting humanity today. Cereal production systems, specifically wheat, rice and maize, account for about two thirds of all energy in human diets. A rising population implies a rising demand for food supply; yet the present rate of increase in yield potential of cereal production does not cover the expected increase in demand (Cassman, 1999). The need is therefore present to search for ways to increase yield potential.

In the past, environmental issues have often been put aside in attempts to increase food production and ensure food security. Intensification of cropping systems during the so called 'green revolution' from the 1950s to the 1970s was based on increased inputs of chemical fertilizers and pesticides, high yielding varieties of wheat and rice and massive investments in irrigation infrastructure (Perkins & Jamison 2008). This form of agricultural intensification enabled an important increase in food production but has had numerous repercussions; soil and water pollution, reduction in the biodiversity of agroecosystems, an effect on the Earth's biogeochemical cycles not to mention the sometimes grave socio-economic implications. The challenge is now to discover how the intensification of agricultural systems can be achieved whilst protecting natural resources and environmental quality for future generations. This goal can be described as **an ecological intensification of agriculture**. It entails improvements in the selection of crop varieties that are efficient in nutrient use, in methods to promote soil fertility, in water use, in energy efficiency and innovations in cropping systems. This also means deepening our understanding of the major nutrient cycles within agro-ecosystems to be able to determine how to keep nutrient cycles in balance.

A recently accepted project at Eco&Sols called Fabatropimed addresses the issue of ecological intensification by examining the ecological services (specifically, symbiotic fixation of nitrogen) of leguminous crops when cultivated in association with cereal crops in tropical and Mediterranean environments. In France the legume-cereal association that has been chosen for study on this project is faba bean-wheat. The faba bean, used worldwide for food and animal feed, displays key ecological benefits for cereal cropping systems. The primary advantages are biological nitrogen fixation and the diversification of the agroecosystem (Kopke and Nemecek, 2009).

This internship aims to study carbon and nitrogen cycles in the agroecosystem of faba-bean and wheat associations. Studying these cycles often implies creating and using models that best represent the transformations and flows of these elements within well-defined parameters of time and space. Models can help illustrate how different agricultural practices affect the cycling of major nutrients and can be used as tools for evaluating and promoting the durability of agricultural systems.

MOMOS (or "Modelling of Organic transformations by Micro-Organisms of the Soil") is a coupled C-N model centred on the activity of microbial biomass that follows the transformations of organic matter through the soil. The main objective of this internship was to work on the application of this model to a field experiment with cultures of faba bean and wheat. The model was proposed (Pansu et al., 2004) then validated (Pansu et al., 2010) from field experiments using  $^{14}\text{C}$  and  $^{15}\text{N}$  isotopic tracers; but has not yet been tested in complex agronomic systems for a short time span (one cultural cycle). During this internship carbon and nitrogen were measured experimentally in the different compartments of the agroecosystem (plants, soil, microbial biomass, release to the atmosphere through measured microbial respiration). Experimental results were then compared with model predictions after corrections had been applied to the model to take into account local climatic factors.

An important component of systems including leguminous plants is the N –fixing role of nodules. Nitrogen fixed by this symbiosis between bacteria and plant roots stimulates plant growth. However, nodules do not yet figure into the production module of the MOMOS model. A complementary aim of this internship is to collect data which could in the future be used to improve the model by extending it to incorporate the role of nodules. The relationship between nodulation and plant growth for faba beans on two sites in the Languedoc-Roussillon region of the south of France will be studied in this perspective.

Time constraint only allows for treatment of data and modelling regarding the carbon cycle. Data collected for the nitrogen cycle will be treated later in time by a PhD student working on the same project on modelling of carbon and nitrogen cycles in France and Tunisia.

This report will first introduce the general context of the internship, then proceed to a general description of carbon and nitrogen cycling and the MOMOS model followed by a description of experimental procedures and results.

## **General Context**

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### ***I. The research unit***

Based on the SupAgro campus of Montpellier, Eco&Sols (Functional Ecology and Biogeochemistry of soils and agro-ecosystems) is a mixed research unit (UMR) linking scientists from SupAgro academic school, the Research Institute for Development (IRD), the National institute for agronomic research (INRA), and the International institute of Agronomic Research for development (CIRAD). The unit is associated with many national, European and overseas partners.

Research aims to study the functioning of plants and soils under the effect of global changes and different agronomical practices. A better understanding of the interactions between plants, soils, soil organisms, and major biogeochemical cycles (C, N and P) within agro-ecosystems is needed to propose agricultural practices that respond to questions of food security whilst simultaneously preserving the environment.

Research at Eco&Sols is organised along three main axes:

#### ***Soils, activities and biological networks***

The research objectives of this section of the UMR are to better understand, describe and use the biological processes that regulate nitrogen and phosphorous dynamics and their bioavailability for plants, C sequestration and the dynamics of biological contaminants. A particular emphasis is placed on the study of the rhizosphere, microbial activity and micro-invertebrates in the soil.

#### ***Nutrients and ecological intensification***

The goal of ecological intensification is to find systems that augment agricultural productivity to satisfy the anticipated increase in food demand all the while meeting acceptable standards of environmental quality. This goal is a challenging one considering the estimates that cereal demand in the next twenty years will raise by 44% for wheat, 43% for rice and 56% for maize

(Cassman, 1999). Meeting this demand without a net expansion of cultivated land to avoid further losses of natural ecosystems requires investigation into alternative forms of production.

At Eco&Sols the alternatives being studied are: the management of organic and mineral inputs, the choice of plant material and associated rhizospheric microorganisms (by the choice and introduction of more adequate genotypes) and the practice of more complex systems such as associated or mixed cultures. Research is carried out on the biogeochemical cycles that characterise the acquisition, use and transfer of nutrients in tropical and Mediterranean agro-ecosystems with low inputs. This entails the study of the stock of nutrients in the soil, their bioavailability for plants, transfer within the plant and recycling in the agro-ecosystem. Modelling of the dynamics of nutrient transfers in the soil/plant system can then be put to the test with data from experimental measurements.

### ***Carbon and global changes***

Carbon is exchanged from terrestrial ecosystems to the atmosphere and vice versa through the processes of photosynthesis and respiration-oxidation. The net balance should be in favour of photosynthesis with carbon being stocked within the ecosystem's biomass and soil organic matter (SOM).

Under this context, the guiding research question that emerges is the following; what factors and major processes within agro-ecosystems determine the production and sequestration of carbon? The approach used to explore this question combines both experimentation and modelling

Identification of the types of land use and management that maximise the ecological service of carbon sequestration helps orient choices towards more durable agro-ecosystems.

## ***II. The research project: FABATROPIMED***

Fabatropimed is the short name for the scientific project: Ecological services of legumes for nitrogen and phosphorous biogeochemical cycles and C sequestration in cereal cropping systems in Africa and the Mediterranean basin. It is directed by Dr Jean-Jacques Drevon, a plant physiologist specialised in leguminous plants at Eco&Sols. This four-year project is still in its beginning phase and collaborators come from a wide array of backgrounds; from local farmers to foreign research partners in Tunisia, Morocco, Burkina Faso and Madagascar. The multidisciplinary, participatory research approach brings in the expertise of specialists in many different areas that integrate experimental field work, lab work, and innovation in developing sustainable agricultural practices and management activities.

The aim of the project is to increase the ecological services of legumes in cereal cropping systems, in other words to increase the benefits that can be pulled from the capacity of legumes to symbiotically fix nitrogen and render it available for plant use. Three areas are being compared: The Mediterranean region (France, Morocco, Tunisia, and Algeria), the Sudano-Sahelian region (Burkina Faso), and tropical regions (Madagascar). In these different environments the efficiency and suitability of cereal cropping systems in association or rotation with grain legumes will be studied.



This study aims to respond to various constraints on existing cereal cropping practices; limited land, water and nutrient availability. Phosphorous is a main limiting factor being studied on this project.

The research on Fabatropimed is divided into 5 work packages with different areas of focus, as can be seen in annex 1. It is principally carried out by a number of PhD projects which are articulated along the lines and objectives of each work package.

This internship contributes to work packages 1 (agronomy, environmental diagnosis and ecology) and 2 (nitrogen and phosphorous cycles, and carbon sequestration in soils).

## **Application of the MOMOS model to a faba bean-wheat agroecosystem: Theory**

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### ***I. Mathematical Models:***

Two types of mathematical models are used when trying to understand the functioning of ecological systems: empirical and mechanistic models.

Empirical (descriptive or statistical) models describe experimental data directly without looking at the functional processes which are associated. A simple linear regression is an example of this kind of model; for example the relationships presented in this report between nodular biomass and aboveground plant production.

Mechanistic models, also known as process-based models, take into account a wider understanding of the links and processes that determine the transport and transformation of elements through an ecosystem. MOMOS falls under this category of model.

It was created using the VENSIM modeling platform as a support which includes a graphic interface for programming the models and visualizing results. Its calculation tools permit the numerical integration of differential equation systems, optimization of parameter values, and sensitivity analyses. It is adapted to the simulation of the dynamics of complex systems in confrontation with collected data bases. The other models TAO and SAHEL used in this study were also programmed on VENSIM

### ***II. Modelling Carbon and Nitrogen Dynamics:***

#### ***A brief description of Carbon and Nitrogen Cycles: Interactions in the soil***

Carbon and Nitrogen are elements found in the basic structure of all life forms, they are cycled through the atmosphere, biosphere, hydrosphere and soil through a multitude of biological,

chemical and physical processes. A large part of organic carbon of terrestrial ecosystems and the main part of organic nitrogen are found in plant residues and soil organic matter. This means that the soil is an extremely important place of storage and exchange of these elements.

Atmospheric carbon enters living forms through plant photosynthetic activity. Plants take in carbon dioxide to create complex carbohydrates that then move into the soil and its microorganisms through root exudates and plant mortality. Microorganisms work on decomposing this organic matter, part of which is easily decomposable, or labile, and part of which remains for a long time as stable organic matter in the soil (becoming a carbon reservoir). The action of these microorganisms converts this organic matter into humus, ammonia and nitrates, available phosphates and micronutrients; which in turn increase the fertility of the soil stimulating plant growth. Simultaneously, root and microbial respiration release CO<sub>2</sub> back into the atmosphere.

Microorganisms also play an essential role in the nitrogen cycle. Atmospheric nitrogen (N<sub>2</sub>) cannot be directly used by plants and therefore passes first through nitrogen-fixing bacteria found in the soil, or in symbiotic relationships on root nodules of leguminous plants. These bacteria, through an energetically costly process, convert N<sub>2</sub> to ammonia (NH<sub>3</sub>) and ammonium (NH<sub>4</sub>) available for plant growth. The action of soil decomposers also breaks down the amino acids and proteins of dead organic matter into ammonium. Then, NH<sub>4</sub> can be further oxidised into nitrates by nitrifying bacteria in the soil, these nitrates are available for plant use and for use by denitrifying bacteria that release N<sub>2</sub> or nitrous oxides back into the atmosphere.

Carbon and nitrogen cycles are closely linked, for this reason the C/N ratio is an important piece of data to collect. Soil microbes are C limited; they depend on plant organic matter as their source of carbon. They tend to have a low C/N ratio which means a large demand for nitrogen per unit of carbon. If plant litter C/N ratios are too high, or higher than 30:1, microorganisms will compete strongly with plants for N liberated during decomposition thus reducing the availability of this N pool to the plants. (Lukac and Godbold, 2011)

### ***Carbon and Nitrogen biogeochemical cycles: Models***

Through modern-day agricultural practices, humans have heavily impacted the earth's carbon and nitrogen biogeochemical cycles. The fundamental discovery of Liebig in last XIX century of absorption of inorganic forms of fertilizers by plant roots has induced extensive synthesis of ammonia from N<sub>2</sub> and H<sub>2</sub>, despite its high energetic cost. This resulted in high increases of crop yield, but current systems have reached limitations and possible inversions from many environmental problems. Nitrogen inputs to terrestrial systems have doubled with careless use of inorganic fertilizers and the cultivation of N-fixing crops (Vitousek et al. 1997). Nitrogen losses from cultivated systems, leading to excess nitrogen within natural ecosystems have caused major environmental problems. Among these are soil acidification (the ammonium ion created with the addition of N fertilizers is a proton donor), eutrophication and surface water toxicity (resulting from nitrate lixiviation) and greenhouse gas release (like N<sub>2</sub>O production during nitrification/denitrification processes or the increase of CO<sub>2</sub> from the respiration of a microbial community unlimited by N stocks) (Gardner & Drinkwater, 2009). Corresponding decrease of the C reservoir can cause other environmental problems such as soil compaction, the decrease of physical fertility, or increase of erosion.

Developing models that closely represent the flow of these elements through the ecosystem can be used as a tool to help evaluate the sustainability of agricultural practices, and guide choices to more suitable forms of crop management. Numerous field studies have shown that crop management

practices can either enhance or diminish quantities of soil C and N, in addition to altering microbial biodiversity over time (Kucharik et al, 2001).

A wide range of models, of different levels of complexity exist to describe soil carbon and nitrogen dynamics. The soil is an extremely heterogeneous environment, and models have to take into account this heterogeneity across different spatial (from the micrometer to thousands of kilometres) and temporal scales (from hours to centuries) (Manzoni and Porporato, 2009). At the moment, there is no universal model that ‘works’ under all conditions.

Most models describing carbon and nitrogen dynamics deal with long-term predictions. However short-term models are important as a tool to address challenges such as achieving high levels of soil organic matter (that increase soil fertility) while keeping concentrations of inorganic nitrogen relatively low during time periods when leaching is at its peak (Petersen et al.). The practical aspect of these models is that they might help to time mineral and organic fertilizer inputs, and other agricultural practices. In the context of current debates on climate change, models can also be used to determine if particular systems are sources or sinks of carbon, and perhaps provide insight into how to maximise carbon sequestration.

### ***III. MOMOS***

MOMOS or “Modelling of Organic transformations by Micro-Organisms of the Soil” is a coupled C-N model that describes the microbial functioning associated to biogeochemical cycling of C and N through the soil and atmosphere. It studies the exchanges between inorganic and organic compartments and determines the fractions of organic C and N forms that remain available for plant production or more stabilized in the soil.

The model is centred on microbial biomass activity and its key ecological functions of decomposition and mineralization. The schema in Fig 1 of the latest version of the MOMOS model illustrates the five compartments (or state variables) that compose the model. Input from the necromass (NC) can be divided into NC stable and NC labile. This quantification will be realised through the application of the TAO model, (later discussed). Both NC materials enter the microbial biomass (MB) compartment which gives labile humus (HL) by microbial mortality; this is then slowly transformed into stable humus (HS) mostly by chemical processes of molecular condensations. Carbon is released back into the atmosphere by microbial respiration (in the model  $qCO_2$  is the metabolic quotient of the microbial biomass).

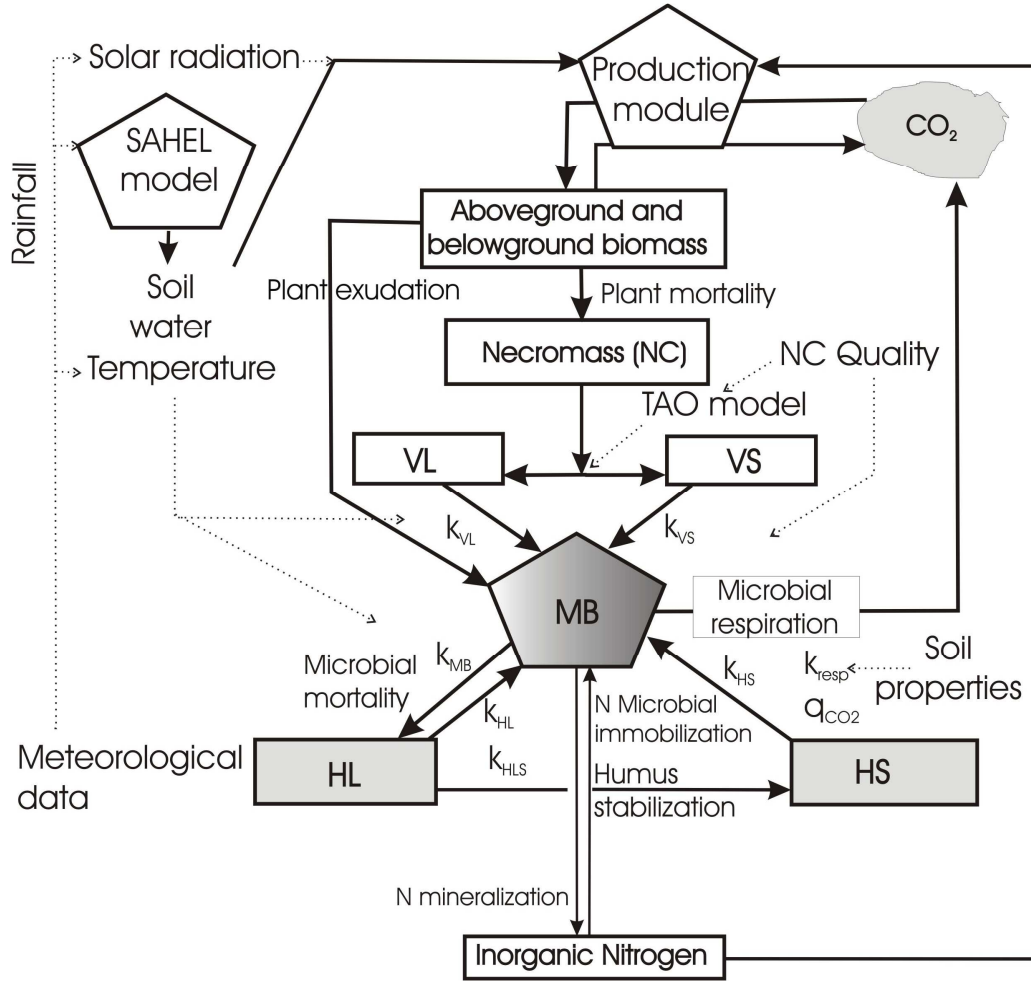


Fig 1 Schema of the MOMOS model, Pansu et al. (2010)

The MOMOS model is sensitive to climate change as all its parameters are influenced by soil moisture and temperature as can be seen in the general equation of the model (Pansu et al. 2010):

$$\dot{\mathbf{x}} = f(T) f(\theta) \mathbf{A} \mathbf{x} + \mathbf{B} \quad (1)$$

where  $\mathbf{x}$  is the vector of the state variables,  $\dot{\mathbf{x}}$  was the corresponding vector of the rate variables,  $\mathbf{A}$  is the parameter matrix of the model,  $\mathbf{B}$  is a vector regulating the external C input by exudation and senescence originated from photosynthesis (Pansu et al., 2009),  $f(T)$  is the response function to temperature correction set to the exponential function:

$$f(T) = Q_{10}^{(T-T_{opt})/10} \quad (2)$$

where  $T$  is the actual daily soil temperature set equal to air temperature;  $T_{opt}$  is the optimal decomposition temperature set at 28°C in Pansu et al. (2010);  $Q_{10}$  is the rate increase when  $T$  increases by 10°C and is fixed at 2.2 in Pansu et al. (2010);  $f(\theta)$  is the response function to soil moisture expressed in fraction of water holding capacity WHC:

$$f(\theta) = \text{MIN}\left(\frac{\theta}{WHC}, 1\right) \quad (3)$$

The soil daily moisture  $\theta$  will be predicted using SAHEL (Penning de Vries et al, 1989) a tipping bucket type model that calculates daily values of soil moisture by layer using climate data (daily maximal and minimal temperature, precipitation and latitude), soil data on water retention and plant cover as inputs.

The model matrix  $\mathbf{A}$  and vector  $\mathbf{x}$  are:

$$\mathbf{A} = \begin{bmatrix} -k_{VL} & 0 & 0 & 0 & 0 \\ 0 & -k_{VS} & 0 & 0 & 0 \\ k_{VL} & k_{VS} & -(q_{CO_2} + k_{MB}) & k_{HL} & k_{HS} \\ 0 & 0 & k_{MB} & -(k_{HL} + k_{HLS}) & 0 \\ 0 & 0 & 0 & k_{HLS} & -k_{HS} \end{bmatrix} \quad \text{and}$$

$$\mathbf{x} = \begin{bmatrix} x_{VL} \\ x_{VS} \\ x_{MB} \\ x_{HL} \\ x_{HS} \end{bmatrix} \quad (4)$$

Where VL and VS represent the labile and stable NC compartments (Fig 1), respectively, MB is microbial biomass, HL and HS are labile and stable humus compartments, respectively.

The model parameters included in the  $\mathbf{A}$  matrix are defined by:

- $k_{VL}, k_{VS}$  the daily assimilation rate of VL and VS compartments by the MB
- $q_{CO_2}$  the metabolic quotient of the microbial biomass,
- $k_{MB}$  the microbial mortality rate
- $k_{HL}, k_{HS}$  the daily assimilation rate of HL and HS compartments by the MB
- $k_{HLS}$  the stabilization rate of HL to HS

At each incubation time, the total C decrease ( $\dot{c}$  from the five compartments  $i \in [VL, VS, MB, HL, HS]$ ) is

$$\dot{c} = \sum_{i=1}^5 \dot{x}_i = -f(T)f(\theta) q_{CO_2} x_{MB} \quad (5)$$

where  $q_{CO_2}$  is the metabolic quotient of the microbial biomass:

$$q_{CO_2} = k_{resp} \frac{x_{MB}}{C_{MB}^0} \quad (6)$$

where  $k_{resp}$  is the respiration coefficient, ( $\text{day}^{-1}$ ) scaled by  $C_{MB}^0$ , the biomass at steady state (estimated on bare soil unsupplied with recent input of substrate).

The use of  $^{14}\text{C}$  and  $^{15}\text{N}$  isotopic tracers of the calibration (Pansu et al., 2004) and validation (Pansu et al., 2010) experiments had the great advantage to simplify the equation 1 system with  $\mathbf{B} = 0$  for

each compartment (no labeled input except at beginning of the experiment) and known initial values for the  $\mathbf{x}$  vector. The challenge of application to the complex system of this work will be to estimate:

- 1) The initial values of the  $\mathbf{x}$  vector in our systems,
- 2) The different inputs in  $\mathbf{B}$  vector at each cropping time from shoot and root materials of each plant, including eventual manual inputs from agronomic management, and C and N importation or exportation from weeds and parasites.

The presented work in this internship is only a beginning to this task. It will be addressed more fully in the 3 years of a PhD thesis that this internship is associated to. The starting point will be values of the  $k$  parameters obtained in the calibration and validation experiments including links between quality of inputs (Bottner et al., 2006) and soil texture regulating microbial respiration (Pansu et al., 2010).

### ***Complementary modelling of soil water using the SAHEL model***

The decomposition of organic matter only takes place under suitable soil conditions, of which soil water content and temperature are the most important factors (Equation 1). Soil water content regulates oxygen levels in the soil. As water content increases, space for air movement within the soil decreases and therefore so does the oxygen supply to the aerobic bacteria which are responsible for a large part of decomposition activity. If the soil is waterlogged, anaerobic bacteria will break down only easily decomposable organic compounds, leaving more plant material to accumulate with its carbon content intact. (Lukac and Godbold, 2011)

In order to estimate soil humidity, MOMOS incorporates predictions from the Sahel model. The Sahel model combines climatic data, in our case collected from the meteorological station of Frejorgues airport, the closest to the experimental site of Manguio with physical soil data to give predictions on the evolution of soil moisture through time. The Sahel model is known to give accurate predictions in semi-arid environments (Penning de Vries and Van Laar, 1982)

### ***Complementary modelling of Transformation of Added Organic Materials using the TAO model***

The TAO (Transformation of Added Organic materials) model is a decomposition model created to describe the transformations of carbon and nitrogen from added organic materials in the soil.

In MOMOS, the TAO model is used to estimate the fractions of labile and stable necromass that then pass through the 'microbial biomass' compartments. Initially the fractioning of added organic material was determined by time consuming incubation experiments. There are alternatives to the incubation method, two of which were tried during this internship; NIRS and the Van Soest procedure of biochemical fractioning of organic matter. These methods will be detailed later on in the report.

TAO-C, which describes carbon transformations, is a parallel three-compartment model using only two parameters: very labile ( $P'_L$ ) and stable ( $P_S$ ) fractions of OM to predict C mineralization. Thuriès et al.<sup>9</sup> showed, using incubation experiments on a wide range of OMs (C:N ratios from 1 to 27) that the mineralization rates of very labile ( $k'_L$ ) and resistant ( $k_R$ ) compartments of AOC could

be considered as constants with  $k'_L = 0.4 \pm 0.15 \text{ day}^{-1}$  (half life  $T_{1/2} = 1.7 \text{ day}$ ) and  $k_R = 0.012 \pm 0.003 \text{ day}^{-1}$  ( $T_{1/2} = 58 \text{ day}$ ). The mineralization rate of the stable compartment [S] was set to 0 for the 6 month experiment. So the residual AOC fraction (RAOCF) after C mineralization was given at time ( $t$ ) after starting time ( $t_0$ ) by:

$$\text{RAOCF} = P'_L e^{-0.4(t-t_0)} + (1 - P'_L - P_S) e^{-0.012(t-t_0)} + P_S \dots\dots\dots (7)$$

where  $(1 - P'_L - P_S)$  corresponded to the fraction of intermediate resistant compounds.

In the 1<sup>st</sup> TAO-C version,  $P'_L$  and  $P_S$  were optimized to minimize the residual sum of squares between observed and predicted data (Powell method). The proposition to link  $P'_L$  and  $P_S$  to biochemical data<sup>12</sup> required first a classification of the OM<sub>s</sub> according to the following  $C_o$  criterion obtained from principal component analysis of the OM set:

$$C_o = 7.18 C_{OM} + 0.14 \text{ Lig}/N_{OM} - 3.84 \dots\dots\dots (8)$$

where C, N, Lig express carbon, nitrogen, and lignin contents in  $\text{g g}^{-1}$  of OM, respectively.

OM<sub>s</sub> with negative  $C_o$  values corresponded mainly to N-rich materials like organic fertilizers or materials of animal origin. Positive  $C_o$  values were attributed mainly to more ligneous materials of plant origin. Equations were then proposed to calculate  $P'_L$  and  $P_S$  according to these  $C_o$  values. The links were then expressed by the following Eq. 7.

$$\begin{aligned} \text{If } C_o \leq 0: P'_L &= 0.35 \text{ fsol} + 2.2 N_{OM} - 0.01 \text{ Lig}/N_{OM}, \text{ and } P_S = 3.60 \text{ Lig} \\ \text{If } C_o > 0: P'_L &= 0.099 \text{ flab} + 0.14 \text{ Hem}, \text{ and } P_S = 1.61 \text{ Lig} + 0.62 \text{ Ash}_{OM} \dots\dots\dots (9) \end{aligned}$$

where  $\text{fsol} = \text{Sol}/(\text{Sol} + \text{Hem} + \text{Cel} + \text{Lig})$ ,  $\text{flab} = (\text{Sol} + \text{Hem})/(\text{Sol} + \text{Hem} + \text{Cel} + \text{Lig})$ ,  $N_{OM}$  was total nitrogen in OM; Sol, Hem, Cel, Lig and  $\text{Ash}_{OM}$  were OM gravimetric fractions obtained by fibre fractionation (Van Soest Method).

## Materials and Methods

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### I. The Study Sites

The two study sites of this internship are both located in the south of France in the Languedoc-Roussillon region as identified on the map, Fig 2. The sites correspond to fields where durum wheat and faba beans are grown both separately and in crop associations.

Considering that the faba bean is not yet widely cultivated in the Languedoc-Roussillon region of the south of France, not many appropriate sites were available. After prospection into farmer's parcels as far away as the Gers, two sites were finally chosen: one based on INRA's experimental site at Mauguio and the other a farmer's field in Camargue.



**Fig 2 Location of study sites, source: [www.geoportail.fr](http://www.geoportail.fr)**

## **Mas Neuf**

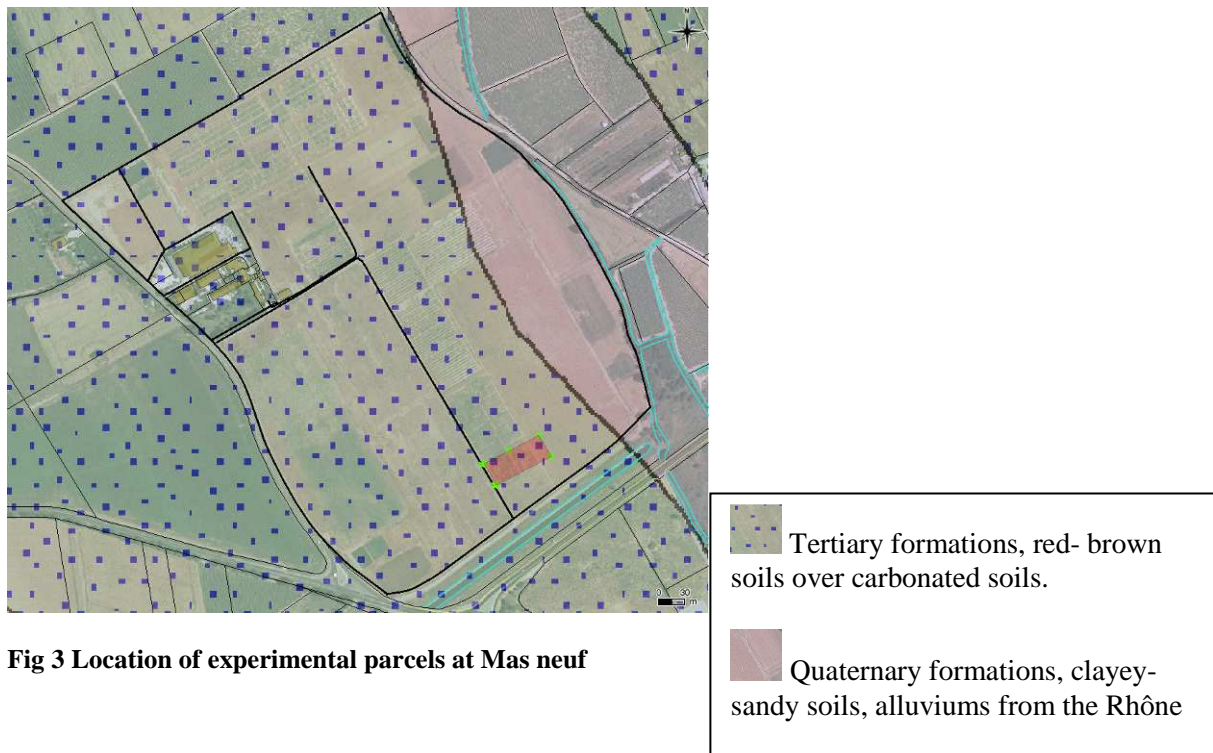
**The main study site is the INRA experimental centre of Mas neuf. As seen in**

Fig 2, the site is located in Mauguio, 15km south east of the city of Montpellier and belongs to the Languedoc plain. A Mediterranean climate and a tendency to water deficiency are characteristic of the area.

The land has belonged to INRA since 1981 and has been used ever since primarily for the cultivation of cereals and leguminous crops. Before 1981 parcels were used for viticulture and fruit production, mainly apples. The site is in its third and final year of conversion to organic agriculture therefore no chemical inputs of fertilizers or pesticides were applied to the parcels.

Soils are generally easily erodible and have a low content in organic matter. Two main areas can be distinguished on the site of Mas Neuf, a zone where sandy-clayey loam soils were formed from alluviums of the Rhône river and another where reddish-brown and carbonated soils of a loamy-clayey texture were deposited over these alluviums. Our parcels, as can be seen on the map in, belong to the latter. The site of Mas Neuf is outlined in black and our parcels (in pink) by green points. A few soil properties are regrouped in Table 1.





**Fig 3 Location of experimental parcels at Mas neuf**

**Table 1 Soil properties of the site of Mas neuf (Ahoulouké, 2009)**

Parameters	Min	Max	Average	St Dev
Ca CO <sub>3</sub> %	0	61,62	7,02	10,28
C <sub>org</sub> (g. kg <sup>-1</sup> )	6,75	21,10	10,22	1,49
N <sub>tot</sub> (g. kg <sup>-1</sup> )	0,55	1,93	0,93	0,13
P <sub>olsen</sub> (mg. kg <sup>-1</sup> )	0,83	64,45	41,26	10,12
P <sub>tot</sub> (mg. kg <sup>-1</sup> )	394	838,51	618,59	65,99
Clay (%)	0	33,03	19,28	4,58
Ltot (%)	29,96	61,85	46,51	6,50
Stot (%)	15,17	54,89	34,24	7,41

## La Rosière

The second site is that of la Rosière, a local farmer's exploitation located in the region of Camargue. The farmer agreed to collaboration with INRA in the spirit of participatory research. This site was used exclusively for the nodular study.

The climate is Mediterranean, and the area is characterised by the presence of strong winds. Soils are sandy-loamy and groundwater has a very high salt content. The presence of salt has defined agricultural practices in the region. Rice production and wheat are often alternated as rice plays the role of desalinating the soil. The region cultivates alfalfa in association with wheat and could benefit from the introduction of more leguminous crops such as the faba bean to ameliorate conditions of cereal production.

## II. Sampling

### *Mas neuf*

The parcels we studied were part of an agronomic field experiment set up by another project in course at Eco&Sols in a random block design with three rows of twelve parcels each (see Annex 1).

Plant and soil samples were collected at various intervals of time in accordance with the crop cycle. Three sampling sessions were carried out at this site. A first session took place three weeks after sowing (March), a second session at the flowering stage of the plants (April) and a final session at harvest time (June) (see Annex 1).

Soil respiration measurements however did not coincide with the dates of our sampling sessions due to the late deliverance of the LICOR machine.

### *La Rosière*

Sampling was between 10 and 20 plants per parcel, at the flowering stage of growth of the faba bean (as it is at this period that symbiotic nitrogen fixation is at its peak).

## **III. Laboratory work**

Modelling carbon and nitrogen cycles in any agro ecosystem requires the movement of these elements to be followed through different parts of the system. This entails a number of laboratory and field experiments followed by subsequent data analysis.

Table 2 regroups the work that was carried out during this internship with respect to its primary objective of studying carbon and nitrogen cycles in a complex system.

**Table 2 Summary table of experimental work**

<b>Model</b>	<b>Laboratory work</b>	<b>Objectives</b>
<b>SAHEL</b>	<ul style="list-style-type: none"> <li>• Soil water content at field capacity</li> <li>• <i>Soil water content at dry (105°)</i></li> <li>• Soil water content at wilting point</li> <li>• Soil cover</li> <li>• Coarse fraction</li> <li>• Collection of meteorological data</li> </ul>	<ul style="list-style-type: none"> <li>• Predictions of soil humidity that can be used in MOMOS</li> </ul>
<b>TAO</b>	<ul style="list-style-type: none"> <li>• NIRS (near infrared spectroscopy) used on plant material</li> <li>• Van Soest method for the determination of biochemical compositions of plant material</li> </ul>	<ul style="list-style-type: none"> <li>• Approximations of the stable component of plant material. The data is then entered into the MOMOS model.</li> </ul>
<b>MOMOS</b>	<ul style="list-style-type: none"> <li>• Plant material: Determination of carbon and nitrogen content using the CHN analyser. Determination of phosphorous content through an attack of nitric acid.*</li> <li>• Microbial biomass: fumigation-extraction method to extract carbon, nitrogen and phosphorous.* <i>Determination of microbial carbon using the TOC.</i></li> <li>• Soils: Determination of carbon and nitrogen content using the CHN analyser. <i>Phosphorous using the Olsen method.*</i></li> <li>• CO<sub>2</sub> field measurements to determine soil respiration rates using the LICOR instrument.</li> </ul>	<ul style="list-style-type: none"> <li>• Compare predicted and measured values for carbon and nitrogen content in plant, soil and microbial biomass.</li> </ul>

\* The experiments regarding composition in phosphorous were carried out under the prospect of being used in the work of a PhD student integrating the phosphorous cycle into the C/N model, and not directly exploited for analysis in this internship.

\* Experimental work in *italics* was carried out by the PhD student working on the same subject, or by one of the lab technicians.

### ***Nodular Diagnosis***

To study the relationship between nodulation and plant growth a protocol developed by Jean-Jacques Drevon was followed in which plant shoot biomass and nodular biomass were measured for a specific site.

Nodules were first counted and then along with the other parts of the plant left for 24hrs in a drying oven at 70°C.

Roots, shoots and nodules of the faba bean plant samples were washed with distilled water and separated.

The shoots and nodules were weighed in order to obtain their dry biomass.



**Fig 4 Faba bean sampling at La Rosière**

### ***Plant Analysis:***

#### **Preparation of samples:**

Plant samples were separated into three parts; shoots, roots and nodules (in the case of the faba bean). Nodules were counted separately, and each part of the plant was dried in the drying oven overnight then weighed. Samples were then ground to a fine powder used in subsequent analyses.

#### **Determination of C and N content:**

The percentage of carbon and nitrogen content in plant roots, shoots and nodules is determined using a CHN analyser. The analyzer uses a combustion process to break down substances into simple compounds which are then measured.

3mg of each sample are measured and placed in a tin capsule that is inserted into the CHN analyser for determination of C and N contents.

#### **Determination of P content:**

Roots, shoots and nodules were first mineralised in mini reactors in a microwave oven with nitric acid, 65%. After mineralisation, the dosage of P content was determined by colorimetry using the yellow vanadomolybdique method. A calibration scale was prepared using a solution with known values of P. Thus the relationship established by the samples of known P content and the absorbance measurements determined by the spectrophotometer allowed to extrapolate the P content of the initial plant samples.

### ***Soil Analysis:***

#### **Preparation of samples**

Soil samples were separated into two parts; one part used immediately for the microbial biomass analysis and the other used for other analyses. Part of the second fraction of the soil samples were ground to 200µm.

#### **Determination of C and N content**

The procedure is identical to the one above described for the determination of plant C and N using the CHN analyser.

#### **Determination of water retention capacity and water retention capacity at wilting point**

Water retention capacity refers to the maximum capacity a soil has to hold water. It can be described as soil water content after the soil has been saturated and gravity has stopped draining the soil. The wilting point corresponds to soil water that is not extractible by plant roots.

These measures were determined according to a procedure based on the difference in mass between soils saturated in water before and after subjection to a certain pressure, as described by AFNOR norms (Pr ISO 11274).

#### **Determination of the coarse fraction of soil samples**

Determination of the coarse fraction of our soil samples was important as texture has an influence both on soil moisture and microbial biomass activity. It thus was a piece of data used in both the SAHEL and MOMOS models.

The coarse fraction was determined by first weighing soil samples, and then passing them through a 4mm sieve. The fraction that doesn't pass through the sieve (mostly comprised of stones) is weighed to give the 'coarse fraction' of the sample.

### ***Microbial Biomass Analysis:***

#### **The fumigation-extraction method:**

Our aim in using the fumigation-extraction method was to determine the amount of carbon and nitrogen contained within the soil microbial biomass. The basic principle of this method is that soil microorganisms die after their cell membranes are attacked by chloroform, and a part of the

microbial constituents, especially in the cyto-plasm, is degraded and transformed into extractable components. (Joergenssen, 1996)

Our soil samples were separated into two fractions one to be fumigated and the other non-fumigated. For the fumigated soils, approximately 10g were measured in petri dishes for C, N and P extractions. These petri dishes were put in a covered bowl with a beaker of chloroform at the bottom for 24 hours, the time necessary for the chloroform to act to break the microbial cell walls. The experiment took place under the laboratory hood for reasons of security.

C, N and P were then extracted from both the fumigated and non-fumigated samples. N was extracted by using a solution of 0,5M  $K_2SO_4$ , C with a solution of 0,05M  $K_2SO_4$ , and P with a solution of 0,5M  $NaHCO_3$ . 30 ml of the extraction solutions were added to each sample. The mixture was then agitated and centrifuged in tubes. The resulting liquid was extracted with disposable syringes and used for subsequent determination of C, N and P quantities.

C content was measured using the TOC (total organic carbon) analyser.

N and P content have not yet been measured due to technical difficulties at the lab.

### ***NIRS Analysis***

Near infrared spectroscopy is a technique that can be used for a large range of practices. It is based on the principle of absorbance of near infrared rays (2500-640 nm) by organic matter. Intermolecular links each have a different vibration which absorbs different wavelengths of the infrared spectra, hence giving each substance it's unique near infrared 'fingerprint' or spectra. To be able to interpret information from a NIRS spectrum a series of reference samples is required in which biochemical laboratory analysis of the composition to be studied have already been performed. This allows for a relationship to be established between biochemical composition and the information presented by a NIRS spectrum.

98 samples of faba bean and wheat were ground, sifted to 200 $\mu$ m (to be sure of the homogeneity in particle size of our samples) and then passed through the NIRS apparatus.

### ***The Van Soest Method.***

This method consists of a biochemical fractioning of our samples by the successive addition of neutral, then acid detergents and finally sulphuric acid. Each step in the process dissolves more and more resistant plant material (water soluble organic molecules, neutral-detergent soluble organic molecules, hemicelluloses, celluloses and a final residual fraction of lignin). By measuring the weight lost at each step in the process it is possible to discover the biochemical composition of the initial sample.

8 samples of faba bean and durum wheat roots and shoots from the different modes of culture were tested using this method.

These experiments were realised at the CIRAD centre, in the laboratories of the research team "Risque Environnemental Lié au Recyclage" with the help and supervision of Jean-Luc Marger.

## ***Soil respiration measurements***

CO<sub>2</sub> production in the soil is mainly a result of root and microbial respiration and is highly influenced by environmental factors such as temperature and rainfall. CO<sub>2</sub> production varies greatly both spatially and temporally, and for this reason accurate soil respiration measurements are somewhat difficult to attain.



**Fig 5 Soil respiration measurement with the Li-Cor 8100A**

Soil respiration measurements were carried out on the field using the Li-Cor 8100A instrument for measuring CO<sub>2</sub> fluxes from soil to atmosphere. Measurements were taken at three intervals of time towards the end of the cultural cycle (due to an unavailability of the equipment earlier on in time). To take into consideration eventual spatial variation of CO<sub>2</sub> emissions within the parcel, 6 measurements were taken (evenly spaced within the parcel) per parcel at each time.

## ***Data Analysis***

For measurements concerning carbon, nitrogen and phosphorous content in plant parts, soil, and microbial biomass standard tests of analysis of variance (ANOVA) using excel were performed to check for significant variation in results between laboratory repetitions and also between field repetitions (the four parcels for each of the three modes of culture). Variation was found to be non-significant in both cases.

Data concerning the nodular diagnosis was treated with R with the help of Didier Blavet and Philippe Deleporte. Linear regression was used to demonstrate the relationship between nodule biomass and plant growth. Analysis of variance was used to check for significant variation in plant growth with respect to mode of culture and nitrogen treatments.

## **Results**

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Only results from carbon experiments and modelling will be addressed in this report as a lack of time and technical difficulties made it impossible to also treat data on nitrogen transformations.

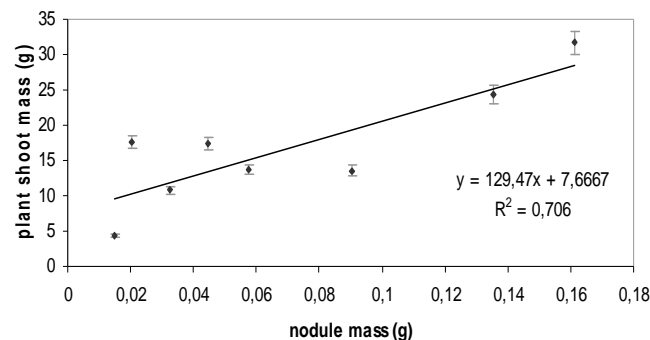
### ***I. Experimental results***

#### ***Nodular Diagnosis***

Complete results for the nodular diagnosis can be found in annex 3, this section will only deal with results from the site of Mas neuf.

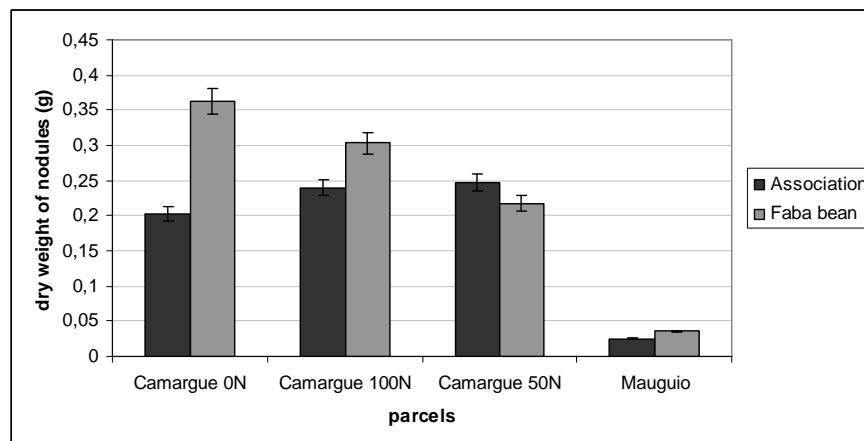
#### ***Relationship between nodulation and plant growth***

Nodule mass and plant shoot mass were found to be positively correlated. However ANOVA results show that the effect of nodulation on plant growth is not significant on the site of Mas neuf. This is a result that differs from the site of La Rosière (see annex 4) where nodule biomass was found to have a highly significant effect on plant growth.



**Fig 6 Relationship between nodular biomass and plant growth, Mas neuf.**

In Mas neuf the relationship between nodule and plant growth is masked by poor experimental conditions. Nodular biomass per plant was low in Mas neuf, less than 0,05g of dry nodules per plant. This can be seen in the comparison of nodular biomass in Mas neuf and La Rosière (Fig 7).



**Fig 7 Comparison of average nodular biomass in parcels in Camargue and Mauguio for associated and non-associated faba beans.**

This lower production can be explained, at least partly, by an attack of sitones (*Sitona lineatus*) and a disease called the “rouille”. The nodules that were measured were not the typical pink, healthy nodules that would be responsible for symbiotic nitrogen fixation, but black empty remains of nodules that had been eaten by the larvae of the sitone (See fig 11). This insect at the larvae stage of development feeds exclusively off the nitrogen-rich nodules of faba bean or pea plants, it then proceeds to feed on the roots and eventually leaves of the plants. Its presence can thus greatly diminish yields. Lower yield is possibly also related to the fact that the terrain in Mauguio is still in the transition phase towards organic agriculture. Yield losses due to nutrient limitation, pest damage, and weed pressure often occur during the transition period from conventional to organic systems following the immediate withdrawal of synthetic fertilizers, insecticides, fungicides, and herbicides (Tu et al. 2006).





**Fig 8 On the left: Healthy nodules on the root of a faba bean collected in Camargue. On the right: Nodules eaten by a sitone, collected from Mauguio.**

Though perhaps not the case in Mas neuf, functioning nodules have a positive effect on plant growth, and therefore on aboveground plant carbon and nitrogen production. It is thus foreseeable as a future perspective to this work to incorporate the nodule function as a factor contributing to plant production, in a plant production model similar to that used in MOMOS.

### ***C/N ratios***

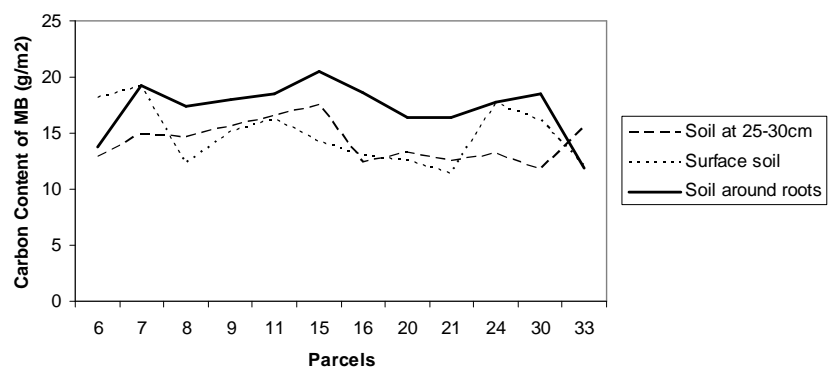
Soil C/N ratios were found to be relatively high for cultivated land. Ratios were often of 12 and above (up to 17) indicating a low capacity for mineralisation which could be due to excessive acidity or anaerobic conditions (Baize, 2000). (See Annex for results)

C/N ratios differed significantly between wheat and faba bean. C/N ratios for wheat ranged from 25-55 at the flowering stage as compared to 15-20 for faba beans at the same stage of development. The return of plant material with high C/N ratios to the soil could diminish the reserves of available N within the soil (Baize, 2000) in turn diminishing microbial activity. Thus it can be justified to interpret that including leguminous crops, of lower C/N ratios (and therefore higher N content) could enrich the soil and enhance microbial activity.

### ***Total soil carbon and microbial carbon***

Measured microbial carbon levels were found to range from 12 to 20 g/m<sup>2</sup>. Total soil carbon ranged from 3.7 to 5.8 kg/m<sup>2</sup>. This means that microbial carbon at approximately 0.4% of total soil carbon is a relatively small proportion and reveals weak microbial activity on our experimental parcels at Mas neuf.

Results revealed a general tendency for microbial carbon levels to be higher in the soil collected around the root area of plants as can be seen in Fig 9.



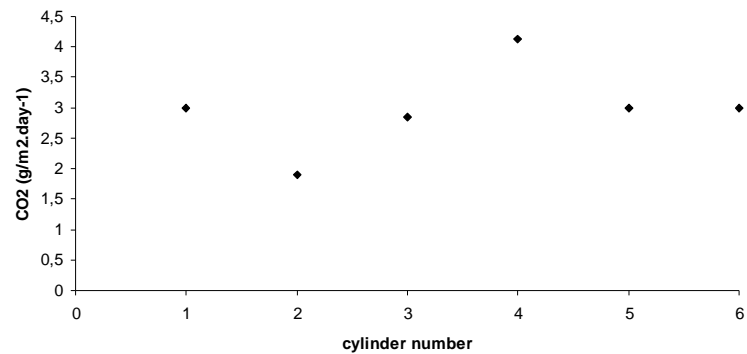
**Fig 9 Experimental values of microbial carbon**



## Soil respiration

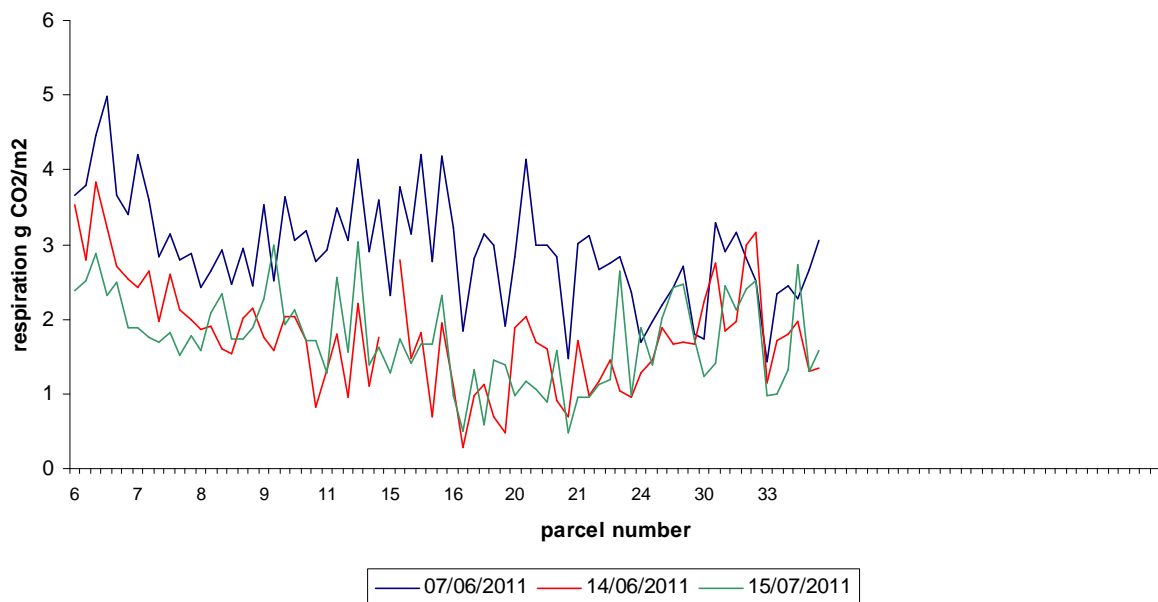
Observations of the few soil respiration measurements taken during this internship indicate a tendency for soil respiration to display both temporal (to the scale of a few hours) and spatial (to the scale of a few m<sup>2</sup>) variation and a sensitivity to environmental factors such as soil moisture.

Within parcels soil respiration was not constant and variation (See Fig 10) was often observed at around 2 g.m<sup>-2</sup>.day<sup>-1</sup> within the 6 measurements taken per parcel.



**Fig 10 Example of intra-parcel soil respiration measurements**

A tendency for soil respiration to decline throughout the day was observed and is demonstrated by Fig 11. Measurements were carried out in the numerical order of the parcels (with parcel 6 being the first and parcel 33 the last ones tested).



**Fig 11 Daily fluctuations of soil respiration measurements, Mas neuf.**

Increases in soil respiration coincided with increases in predicted soil moisture as demonstrated by Fig 12 which plots soil respiration and soil moisture measured in a faba bean- wheat parcel. Similar trends were observed in the other parcels.

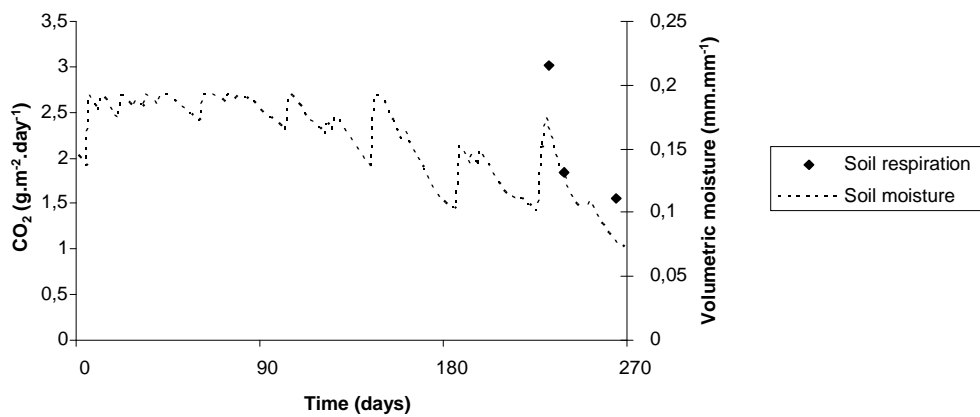


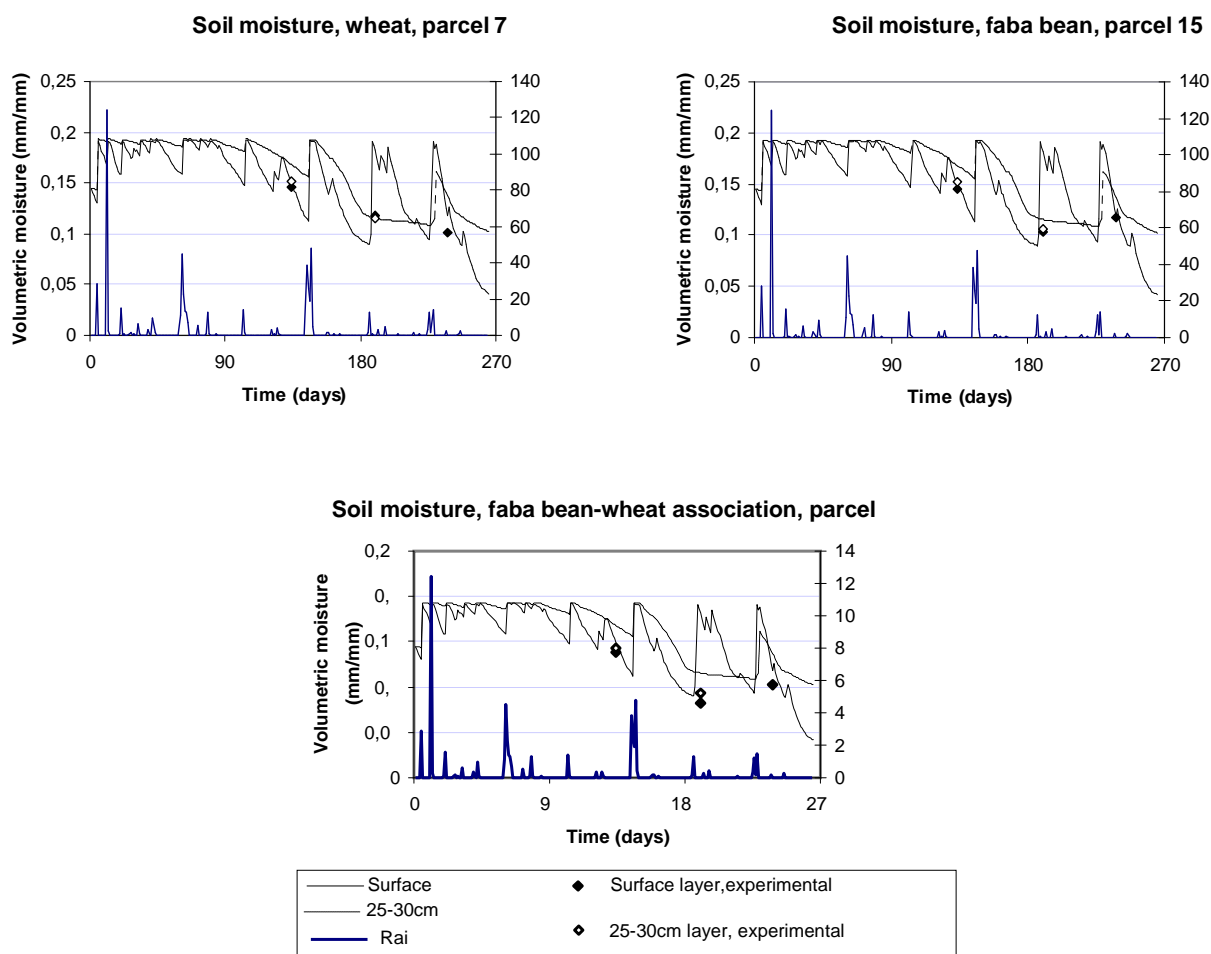
Fig 12 Soil respiration and soil moisture evolution over time (under faba bean-wheat association), see below estimation of soil moisture (dotted line) by the SAHEL model

## II. Modelling Results

### *Estimations of Soil Moisture; SAHEL*

The results obtained from the Sahel model were quite satisfactory in comparison to the experimental data we obtained from soil moisture determination. The model seems to accurately predict the evolution of soil moisture, taking into account a range of atmospheric and soil factors. The following figures show the close correlation between our experimental points and Sahel estimations of the evolution of soil moisture in two soil layers (at the surface and at a depth of 25-30cm).

Peaks in soil moisture, especially in the upper layer correspond with periods of heavier rainfall. The lower layer varies less in function of rainfall.



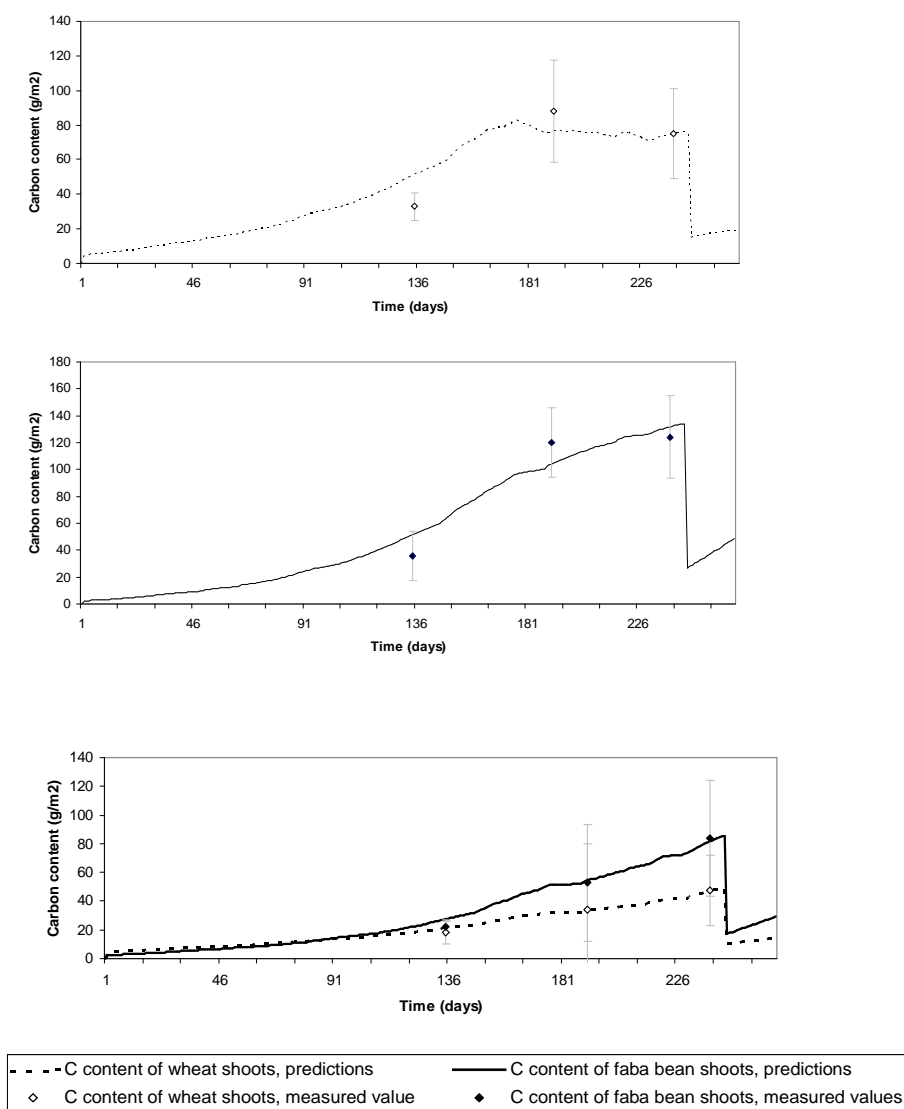
**Fig 13 Measured values and predicted tendencies of soil moisture (as simulated by SAHEL) in two upper soil layers**

A possible explanation for the slight discrepancies observed between experimental and predicted soil moisture values is that climatic data was not collected directly on the experimental site. Although the weather station at the airport of Frejorgues (see Fig 2) was only approximately 10km from our site, a closer collect of climatic data could have resulted in more precise predictions.

No significant differences were observed in soil moisture between the three different cultural practices (wheat, faba bean and the association wheat-faba bean) either in predicted or measured values.

### ***Carbon production and transfers in faba bean and wheat plant organs***

Measured values coincided closely to predicted values of carbon by the plant production module of MOMOS (Fig.3 and annex 4) content in wheat and faba bean shoots, roots and grains as can be seen in Fig 14.



**Fig 14 Predictions and measured values, with their intervals of confidence, of carbon content in plant shoots in wheat (top), faba bean (middle) and associated wheat-faba bean (bottom) parcels.**

It can also be observed that carbon production of faba bean and wheat crops is higher when crops are grown individually than in association. However, the combined carbon production of faba bean and wheat crops in the association gives a higher carbon plant shoot content per m<sup>2</sup>. Carbon content in plant aboveground production rises to about 90 g/m<sup>2</sup> for wheat parcels, approximately 120 g/m<sup>2</sup> for faba bean parcels and approximately 130 g/m<sup>2</sup> for the association.

Values for the transfer rates of C from shoots to roots and grains, for growth rates of plant organs and mortality rates of the plant roots and shoots were optimised with the corrective factor 'fcor' based on temperature and humidity.

Modelling of carbon production and transfer through plant components allows estimation of the necromass that enters the carbon decomposition model. The necromass has 4 origins; faba bean and wheat roots (determined by the root mortality rate) and shoots (where the main input is at harvest time with the reincorporation of approximately 80% of plant shoot production).

### ***Estimations of stable and labile plant components:***

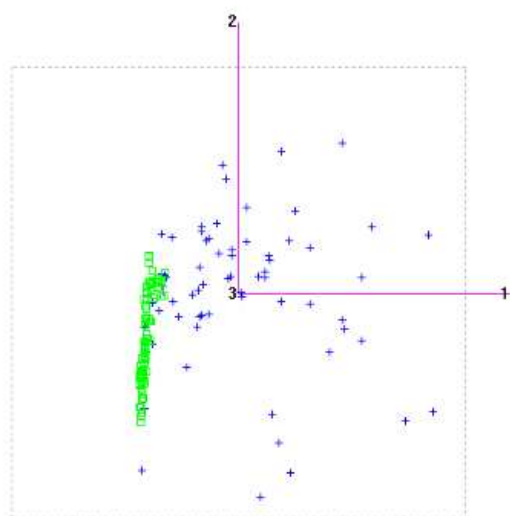
Estimating stable and labile plant components by the TAO model without passing through lengthy incubation experiments (of at least 6 months long) was a challenge that required a bit of trial and error:

#### ***NIRS:***

Our NIRS results were not exploitable for estimating the stable and labile fractions of plant samples. The reason behind this lay in the incompatibility of our samples with the reference samples available.

As a reference we had planned to use the work of Kaboré et al, who had already established the relationship between NIRS results (or spectres) and the biochemical composition of a large range of organic substances. His samples were of a highly heterogeneous nature (from animal manure to compost to urban waste). Our samples were comparatively homogeneous in nature, and only of plant origin.

When the two data sets of NIRS spectras from both sample sets were compared, it was found that there was not a close enough relationship, only 18 out of 98 samples were comparable to Kaboré's data. The Principal components analysis in Fig 15 illustrates the incompatibility of the datasets.



**Fig 15 Principle components analysis showing spectras from Mauguio (green) and reference spectras from Kaboré et al (blue).**

Thus using the relationship Kaboré et al (2011) established between NIRS and biochemical composition would have given erroneous results for our samples. We then turned to the option of using the Van Soest method.

#### ***Van Soest:***

The Van Soest method gave us the results detailed in Table 3. The acquired data on soluble material, hemi-cellulose, cellulose and lignin content of our plant samples enabled us to determine their labile and stable fractions using as reference the work of Thuriès et al. (2002)

**Table 3 Results of the Van Soest experiment; biochemical composition of Faba bean and Durum wheat.**

Mode of Culture	Crop	Soluble	Hemi-cellulose	Cellulose	Lignin	Ash
Association	Faba bean, shoots	49,59	10,55	17,72	9,43	12,70
Association	Faba bean, roots	52,19	9,90	21,85	7,25	8,80
Monoculture	Faba bean, shoots	48,84	12,83	17,06	9,65	11,61
Monoculture	Faba bean, roots	51,97	9,91	20,48	7,05	10,58
Monoculture	Wheat, shoots	38,78	26,74	22,79	1,90	9,80
Monoculture	Wheat, roots	31,07	23,57	22,74	5,31	17,30
Association	Wheat, shoots	38,76	26,04	20,01	4,32	10,88
Association	Wheat, roots	28,39	27,17	23,99	5,23	15,22

The Van Soest data, together with data of the carbon and nitrogen contents of the plants, (calculated previously on the CHN analyzer) were used in the TAO model to calculate the following labile and stable fractions, displayed in Table 4.

These calculations were realized with formulas established by Thuriès et al. Considering that our samples were all of plant origin we used the equations corresponding to positive Co values (Ec 7).

**Table 4 Stable and labile fractions of plant material, as determined by TAO.**

Mode of culture	Plant	Plant Part	Labile fraction	Stable fraction	Resistant fraction	C/N
Association	Faba bean	Shoot	0,0829	0,2306	0,4435	16,8735
Association	Faba bean	Root	0,0812	0,1713	0,7473	31,2560
Monoculture	Faba bean	Shoot	0,0870	0,2274	0,4436	17,2932
Monoculture	Faba bean	Root	0,0824	0,1792	0,5527	26,8063
Monoculture	Wheat	Shoot	0,1093	0,0913	0,7812	45,1728
Monoculture	Wheat	Root	0,0984	0,1928	0,7087	67,1677
Association	Wheat	Shoot	0,1084	0,1369	0,6992	33,6495
Association	Wheat	Root	0,1029	0,1786	0,7189	69,6482

We also observed that there were no significant differences between results according to mode of culture, and therefore used averages values in MOMOS.

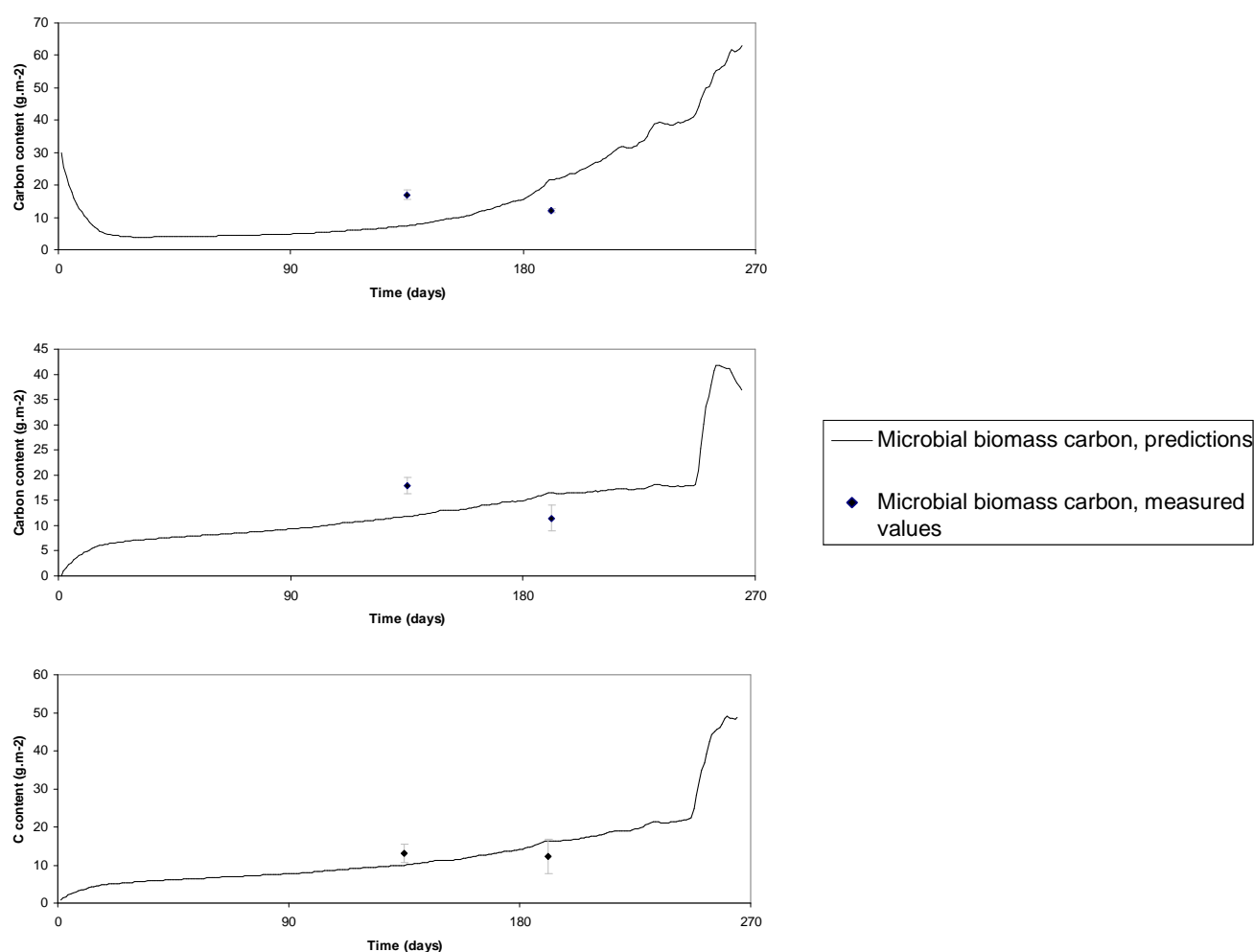
Not having performed incubation experiments on our samples, means that it is impossible for us to compare measured and predicted values of labile and stable carbon fractions. The assumption is therefore made that the relationships established by Thuriès are accurate in our case also

## ***Modelling Carbon transfers from plants to micro-organisms during one cropping season using MOMOS***

Parameter values of the MOMOS-C model previously established in works by Pansu et al and Bottner et al. were optimised to take into account climatic corrections based on temperature and soil moisture. (See annex 4)

### ***Microbial Carbon***

Measured values of microbial carbon correspond best to predicted values in the associated parcels as can be seen in Fig 16.



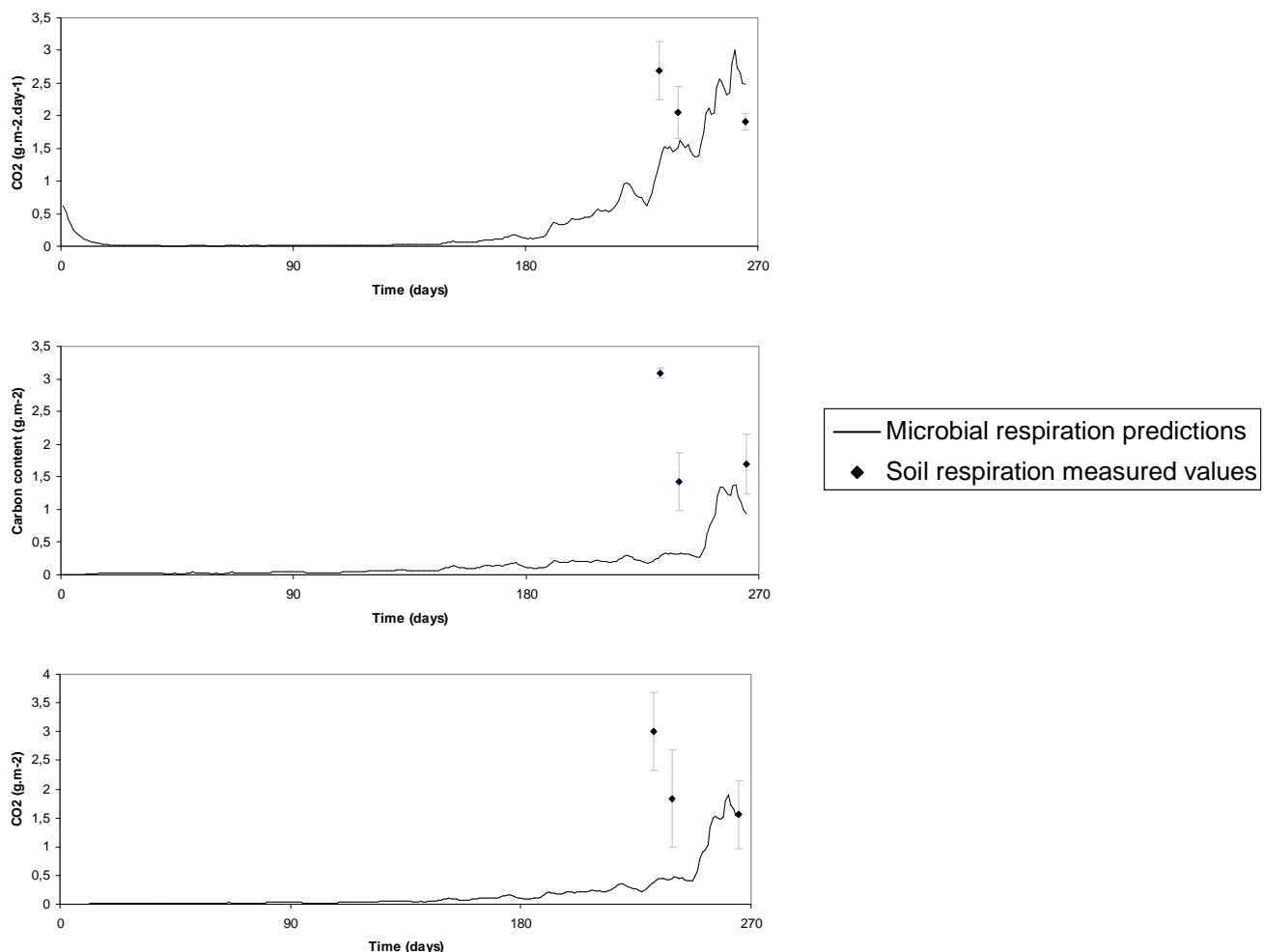
**Fig 16 Microbial biomass carbon predictions and measured values, with their intervals of confidence, under wheat, faba bean, and associated wheat-faba bean parcels respectively.**

Model predictions indicate an important rise in microbial biomass carbon towards harvest time. This rise in microbial biomass is parallel to an increase in inputs of necromass from crop plants at the end of their life cycle. It can be observed that the microbial biomass is thus sensitive to changes in its environment and responds quickly.

Although levels are generally low in all experimental parcels, microbial biomass carbon rises to higher levels in wheat parcels than in faba bean parcels. This is surprising as wheat has a higher C/N ratio than the faba bean. Once reincorporated into the soil plant material with high C/N ratios tends to diminish available N for microbial activity, so one would expect the reincorporation of faba bean residues to stimulate microbial activity more than wheat.

### *Microbial Respiration*

Microbial respiration measured values were not always close to predicted values. This is especially the case for the first measurement of soil respiration on the field.



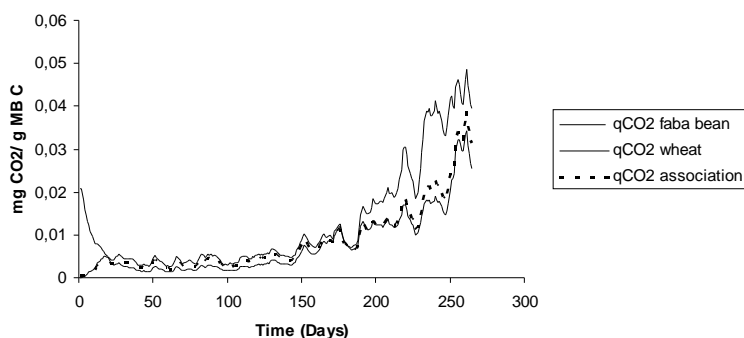
**Fig 17 Predicted and measured microbial respiration in parcels of wheat, faba bean and wheat-faba bean associations respectively.**

Microbial respiration predictions reach higher values in wheat than in faba bean parcels. Respiration however is faster in faba bean parcels, as can be interpreted from the steep rise in respiration rates towards harvest time as opposed to a more gradual increase in wheat parcels. C/N



ratios may explain this observation, as a higher C/N ratio for faba beans implies that microbial communities are not limited in N.

The metabolic quotient of the microbial biomass, expressed as mg of CO<sub>2</sub> emitted per g of microbial carbon, is an indicator of the activity state of the microbial biomass. In wheat parcels the predicted metabolic quotient is higher than in faba bean and associated parcels.



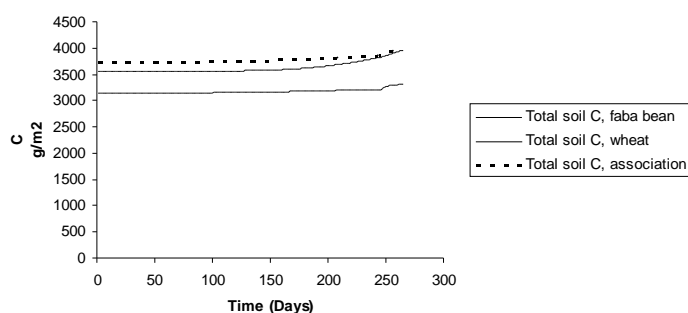
**Fig 18 Metabolic quotient, qCO<sub>2</sub>, in faba bean, wheat, and faba bean-wheat parcels**

Soil respiration is composed of both autotrophic respiration (plant roots) and heterotrophic respiration (soil microorganisms). Field measurements did not separate these two components. Predicted values estimated only the heterotrophic component. It is therefore to be expected that measured values for microbial respiration be higher than predicted values. The last measure of soil respiration was taken ten days after harvest time, when root respiration can be considered minimal, and this value is the one that comes closest to MOMOS predictions.

The contribution of plant root respiration to total soil respiration can vary greatly, it has been estimated in literature at varying percentages; 45-60% (Hanson et al, 2000), 70-80% (Bowden et al, 1993), 56% (Tang et al, 2005), 60% (Epron et al, 1999) under different types of vegetation.

If we consider MOMOS predictions of microbial respiration as accurate, we can very roughly estimate the contribution of plant root respiration (by subtracting predicted microbial respiration from measured soil respiration) to about 40% for wheat and 80% for faba beans.

### *Total Soil Carbon and the net Carbon balance*

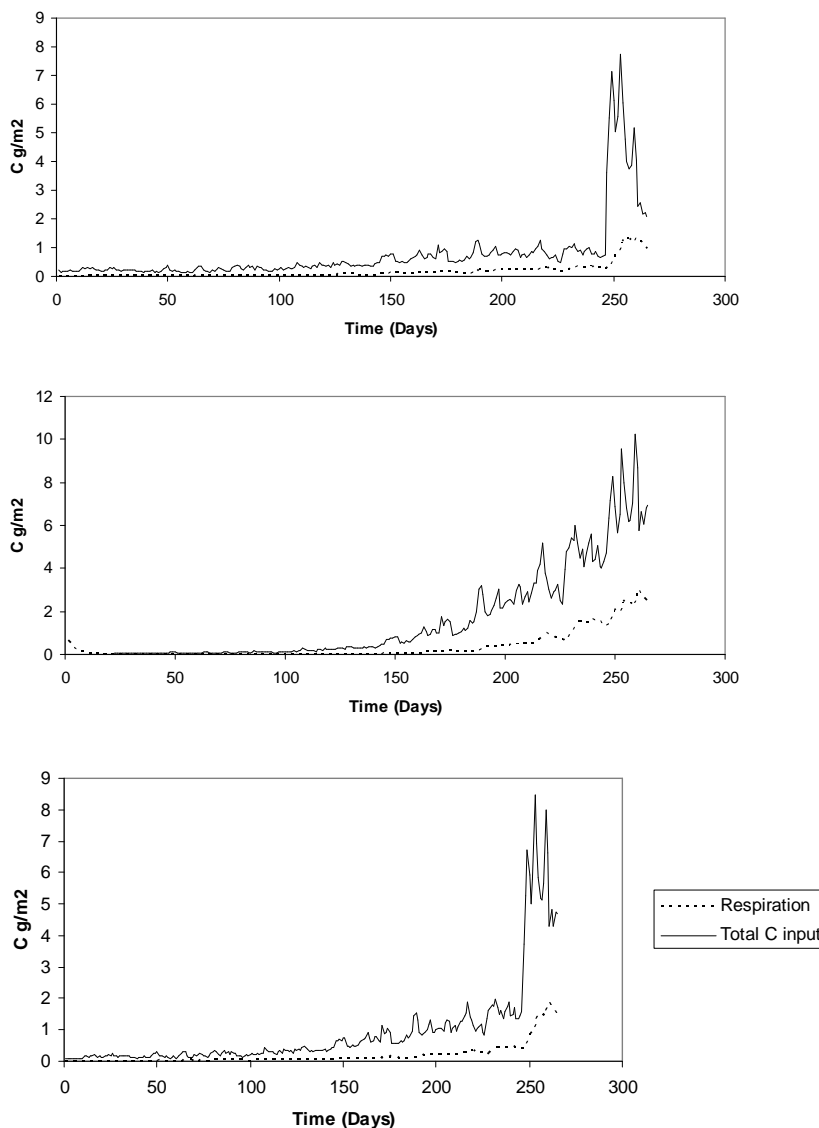


Total soil carbon in all three parcels augments slightly towards the end of the cultural cycle towards harvest time. Indicating that more carbon enters the system than leaves it.

**Fig 19 total soil carbon predictions in faba bean, wheat and associated parcels**

Plotting microbial respiration against total plant input of C allows us to form an idea of if the agrosystem in question has a tendency to act as source or sink of carbon. Microbial respiration represents the exit of C from the system, and plant material necromass is the entrance. It can be observed in Fig 20 that carbon input is higher than output through microbial respiration and it can be inferred that all three of the studied modes of culture act as carbon sinks.

However in this case, we have also noted that stable humus decreases slightly over time as the labile pool increases. As stable forms of C are transformed to labile it leads to question the durability of the system. Though it seems to stock carbon in the short term (of one cultural cycle) the behaviour of this system remains to be studied when plant input is reduced (for example during the winter).



**Fig 20** The carbon balance; total carbon input from plants and carbon output from microbial respiration in faba bean (top), wheat (middle) and associated (bottom) parcels.

## Discussion

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Microbial biomass activity on the study site of Mas neuf has been observed to be quite low (microbial carbon is at barely 0.4% of total carbon) indicating poor soil quality. A decrease in microbial carbon means a decrease in microbial mortality and thus a decrease in the labile humus pool which is a potential nutrient source for plants. In organically managed agrosystems, the microbial biomass dominates the nutrient supply through mineralization processes as synthetic fertilizers are non existent (Tu et al, 2006). The relatively small microbial community at Mas neuf is possibly one of the contributing factors to poor crop yield.

One of the abiotic factors that most affects microbial biomass is soil texture (Pansu et al, 2007). It has been demonstrated that clay content has a positive relation to size of the microbial biomass community (however it reduces respiration activity as the reduced amount of pore space in the soil reduces air passage, and risks becoming waterlogged and reducing aerobic activity). The soil at Mas neuf is low in clay content, which is at approximately 19% (Aholoukpé H., 2009) and therefore texture could be one of the factors explaining the low microbial community.

In literature, it has been attributed to the microbial biomass the capacity of being a good indicator of soil quality due to its rapid response to environmental change. Model predictions and experimental results affirm microbial sensitivity. Peaks were observed straight after rainfall, or increases in soil moisture. Microbial biomass carbon, and its respiration, increase in parallel to inputs of carbon to the soil from plant necromass.

Surprising is the result that microbial biomass carbon content is lower in faba bean parcels than in wheat parcels. Generally, the fact that faba bean plants have a lower C/N ratio than wheat would lead to the hypothesis that this leguminous plant would stimulate microbial growth more than wheat would.

The poor field conditions at Mas neuf seem to have masked what would have been the expected ecological benefits of the faba bean. Poor nodulation at the flowering stage of the plants due to insect attack implies less symbiotic nitrogen fixation, and therefore a loss of one of the great advantages of leguminous plants. Nodulation in healthy plants is highly correlated with plant growth (example of Camargue in Annex 3) and therefore impacts the carbon input to the MOMOS model.

### *Future Perspectives*

Ecological modelling is based on the principal of simplifying what is observed or measured in the natural world to extrapolate trends, relationships, causes and effects to ameliorate our understanding of ecological systems and eventually permit predictions of likely future tendencies. However the natural world is full of surprises and one of the challenges of this internship was taking into account all the surprise factors. For continued work on applying MOMOS to a complex system and if parcels are to be used in the same place for a continued study next year it could be interesting to address the following problems we encountered this year:

- Plant health: sitones and rust for faba beans
- Weed management
- A more regular set up for measuring soil respiration.
- Proper study of the fraction of autotrophic respiration in total soil respiration

- Continued study of carbon stocks and microbial activity on the same parcels throughout the year to have a more complete picture on the durability of the modes of culture studied

An analysis and completion of data collected with regards to the nitrogen cycle would be an interesting complement to the work presented in this report, as carbon and nitrogen cycles are closely linked.

## Conclusion

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The work realised in Mediterranean conditions during this internship was a first step in applying MOMOS to a complex system. Comparing model predictions to a whole range of field collected data gave overall encouraging results which showed that the model is able to simulate carbon transformations and movements from plant to soil and atmosphere, based on the parameters defined in previous  $^{14}\text{C}$  calibration and validation experiments in tropical conditions.

This internship touched upon a wide range of factors that play a role in the carbon cycle, and observations of patterns, relationships and trends lead to questions for eventual further study:

- ❖ How can nodules be incorporated into the MOMOS plant production module as a factor stimulating plant growth?
- ❖ What are the factors that could lead to an observed increase in microbial biomass of wheat parcels as opposed to faba beans?
- ❖ What is the contribution of heterotrophic respiration to total soil respiration?

The difficulties encountered in this internship regarding crop health can serve as a basis for reflection for further study into the application of MOMOS to complex systems.

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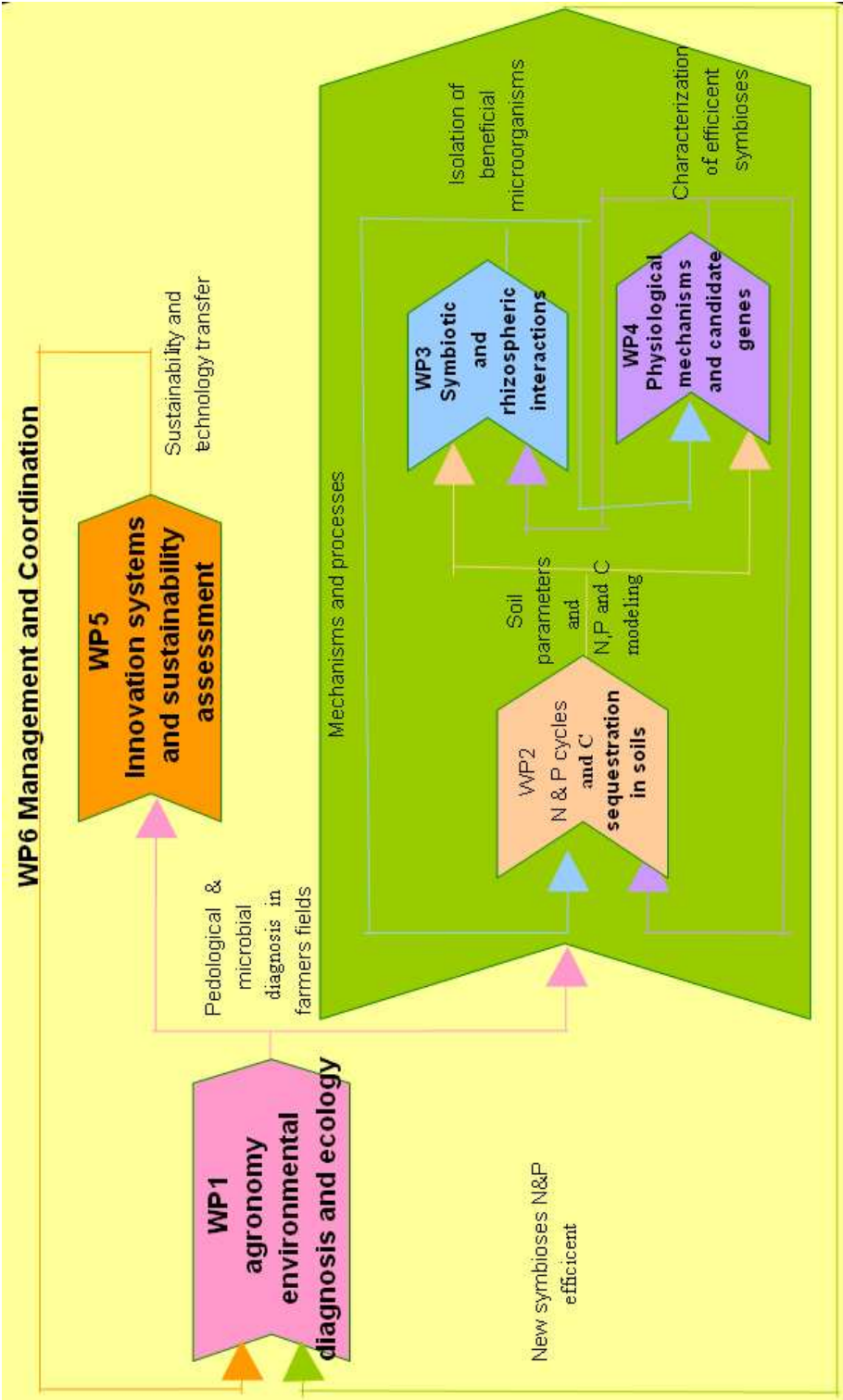
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Annexes

Annex 1: Organisation of the different work packages on the project FABATROPIMED



## Annex 2: Experimental field layout and sampling schedules:

### Mas Neuf

The following schema highlights the parcels that were studied during this internship; in green: faba beans, in yellow: wheat, and in pink: the association of faba bean and wheat. The other parcels were comprised of chickpea and another variety of wheat.

					Associa tion	Durum wheat	Durum wheat	Faba bean		Faba bean	
		Faba bean	Faba bean				Associa tion	Associa tion			Durum wheat
					Durum wheat			Associa tion			

The below table details plant and soil sampling sessions:

Date	Plants	Soil
02/03/11	<ul style="list-style-type: none"> <li>2 plants per parcel.</li> </ul>	<ul style="list-style-type: none"> <li>3 soil samples per parcel; soil is sampled at the surface, at a depth of 25-30cm and around plant roots.</li> </ul>
27/04/11	<ul style="list-style-type: none"> <li>10 plants per parcel + samples of the weeds that are present in the field.</li> </ul>	<ul style="list-style-type: none"> <li>3 soil samples per parcel; soil is sampled at the surface, at a depth of 25-30cm and around plant roots.</li> </ul>
14/06/11	<ul style="list-style-type: none"> <li>2 plants per parcel.</li> </ul>	<ul style="list-style-type: none"> <li>3 soil samples per parcel; soil is sampled at the surface, at a depth of 25-30cm and around plant roots.</li> </ul>
Total:	168 plants+weeds	108 soils



### Annex 3: Nodular Diagnosis of the Faba Bean (*Vicia Faba*)

A nodular diagnosis studies the nodulation of a legume in a given area of production relating it to plant growth and yield. The objective is to determine whether there is a significant relationship between plant growth and symbiotic nitrogen fixation.

Leguminous plants have the capacity to fix atmospheric nitrogen by forming a symbiotic relationship with bacteria in the soil. These bacteria attach themselves to the roots of leguminous plants and form what are known as nodules, the sites where fixation of N occurs. Symbiotic bacteria, in the case of the faba bean, *Rhizobium*, are able to reduce N<sub>2</sub> to ammonia because they are shielded from free oxygen by the host plant; the main fixation enzyme, nitrogenase, is inhibited by free oxygen. Legohaemoglobin regulates the internal oxygen concentration in nodules (Lukac and Godbold, 2011). It is also what gives properly functioning nodules their pinkish colour.

In the framework of Fabatropimed, this nodular diagnosis was carried out with the objective of selecting farmer's fields that could be used in further studies for the project. Two sites were chosen; Mas Neuf and La Rosière (Camargue).

The cultural precedents on the site of La Rosière were wheat, and before that rice. A treatment was applied to the parcels against disease (fusariose), and nitrogen fertiliser was applied to different degrees across three bands in the parcels. The top band received 4 inputs of nitrogen (2 inputs of urea and 2 of ammonium) and a total of 229kgN/ha. The lower band received 104kgN/ha in two inputs (1 urea and 1 ammonium). The middle band was free from nitrogen fertiliser.

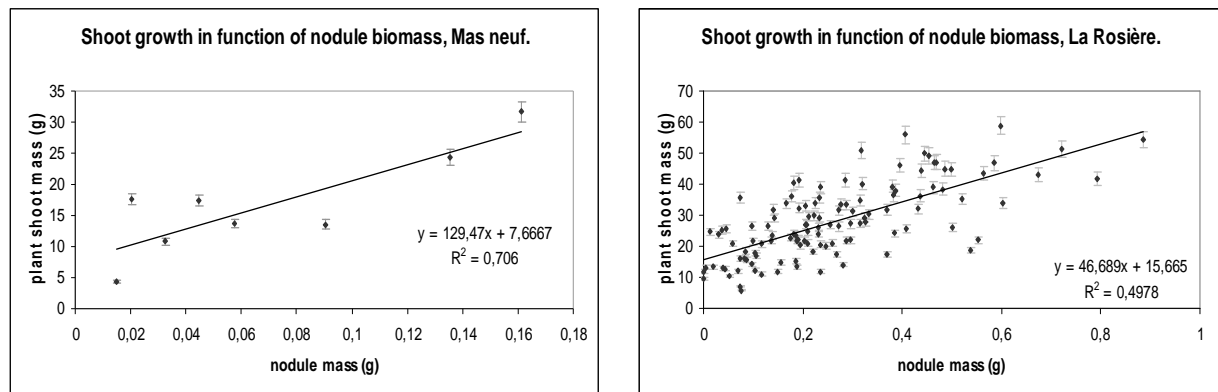
104 kg N/ha	0 kg N/ha	229 kg N/ha						<b>Faba bean-durum wheat</b>	<b>Faba bean</b>
								<b>Faba bean-durum wheat</b>	<b>Faba bean</b>
								<b>Faba bean-durum wheat</b>	<b>Faba bean</b>

The experimental set up of this nodular diagnosis allowed analysis of: Firstly, the relationship between nodule biomass and plant growth on both sites. Secondly, the effect of mode of culture, or the association faba bean-durum wheat, on plant growth on both sites. Finally the impact of different levels of nitrogen input on the nodule/plant growth relationship.

### *Relationship between nodulation and plant growth; as observed in Camargue and Mauguio*

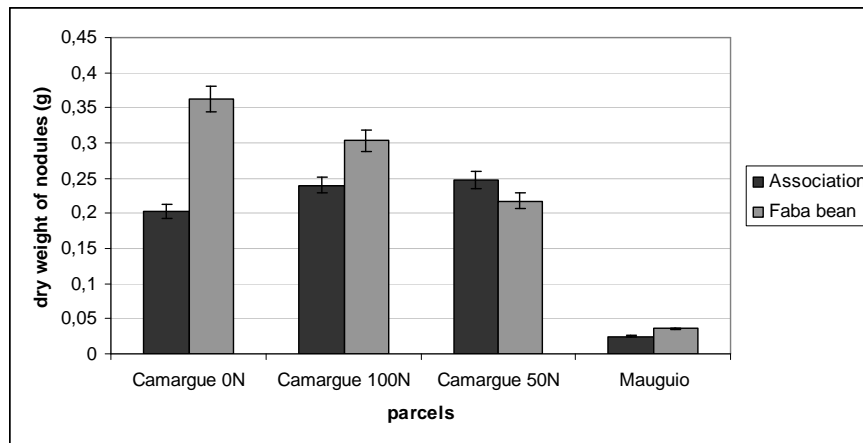
Data was analysed using standard analysis of variance (ANOVA) tests using R (version 2.13.0). Results concluded that although the effect of nodule biomass on shoot growth was highly significant on the site of La Rosière (see fig....), no significant effect was observed on the site of Mas neuf.

In Camargue, as can be seen in fig 1, the relationship between nodule mass and shoot mass can be described as linear. An increase of 1 g of nodule biomass corresponds to an increase of about 47g of plant aboveground production.



**Fig 21 Shoot growth in function of nodule biomass in faba bean plants, Mas neuf and La Rosière**

In Mas neuf the relationship between nodule and plant growth is masked by poor experimental conditions. Average nodular biomass in Mas neuf was significantly lower than in La Rosière (Fig.). This lower production can be explained, at least partly, by an attack of sitones (*Sitona lineatus*) and a disease called the “rouille”. The nodules that were measured were not the typical pink, healthy nodules that would be responsible for SNF, but black empty remains of nodules that had been eaten by the larvae of the sitone. This insect at the larvae stage of development feeds exclusively off the nitrogen-rich nodules of faba bean or pea plants, it then proceeds to feed on the roots and eventually leaves of the plants. Its presence can thus greatly diminish yields. Lower yield is possibly also related to the fact that the terrain in Mauguio is still in the transition phase towards organic agriculture. Yield losses due to nutrient limitation, pest damage, and weed pressure often occur during the transition period from conventional to organic systems following the immediate withdrawal of synthetic fertilizers, insecticides, fungicides, and herbicides (Tu et al. 2006).



**Fig 22 Comparison of average nodular biomass in parcels in Camargue and Mauguio for associated and non-associated faba beans.**

*Relationship between the mode of culture; association or non-association on plant growth, Camargue and Mauguio.*

When nodule biomass is taken into consideration it masks the effect of the mode of culture on plant growth in Camargue and Mauguio. However when nodular biomass is not taken into account, mode of culture seems to have an important effect on plant growth in Camargue and a lesser effect in Mauguio. The tendency observed is that the non-association favours plant growth of the faba bean.

*Effect of Nitrogen fertilizer on plant growth and nodule formation.*

The effect of nitrogen fertilizer can be observed only on the experimental site of La Rosière. ANOVA tests show that N fertilizer has no significant effect on faba bean plant growth. A possible explanation could be that symbiotically fixed N from plant nodules covers the plants needs and therefore additional N input does not make a significant difference on plant growth (at the three levels tested in Camargue).

Summary of results of the Analysis of Variance:

Camargue	Effect of nodule biomass on plant growth	***
	Effect of mode of culture on plant growth	***
	Effect of N fertilizer treatments on plant growth	
	Effect of N fertilizer treatment on nodule biomass	
	Effect of mode of culture on nodule biomass	***
Mauguio	Effect of mode of culture on nodule biomass	.
	Effect of nodule biomass on plant growth	
	Effect of mode of culture on plant growth	.

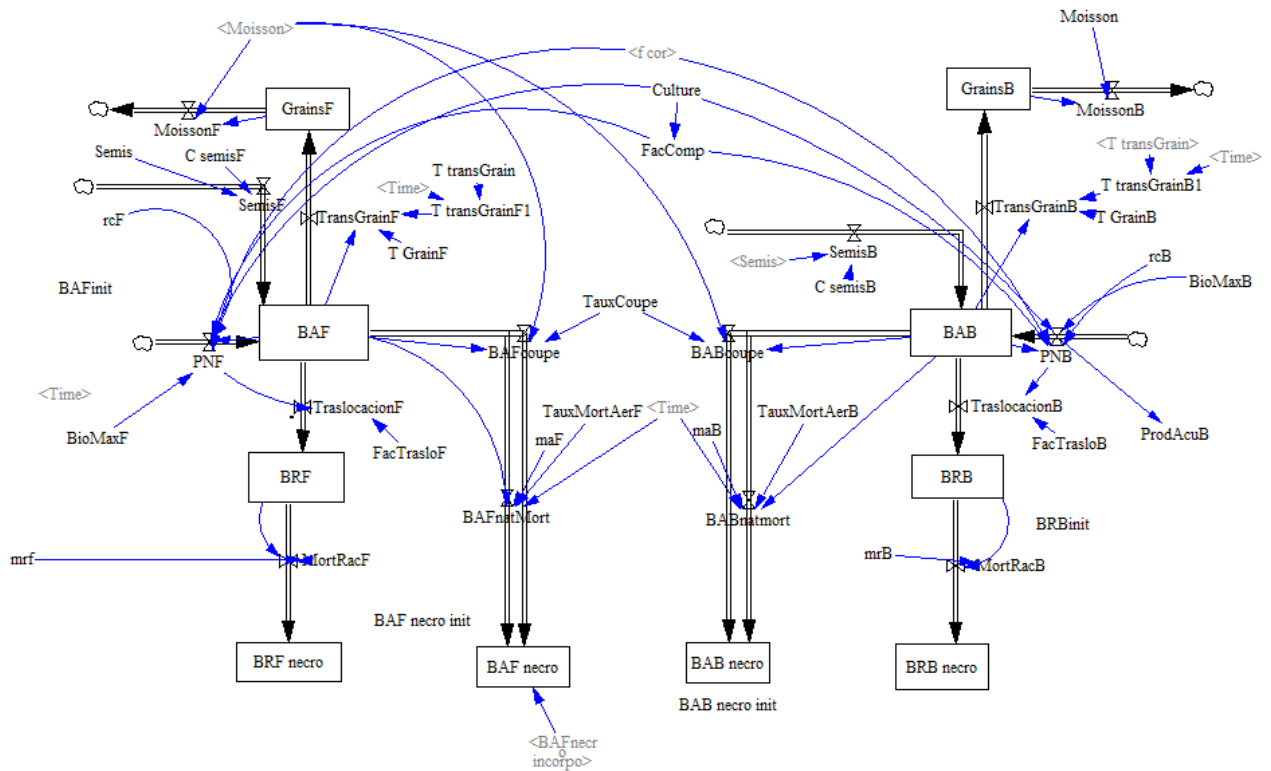
Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Annex 4a:** The plant production module used in MOMOS as modified on VENSIM for use in Faba bean - Wheat systems:

BAF is C aerial biomass of fababeau, BRF is C root biomass of fababeau, GrainsF is C grain biomass of fababeau, MortRacF is daily root mortality of BRF, BAF necro is C from fababeau litter brought both by natural mortality and human intervention,

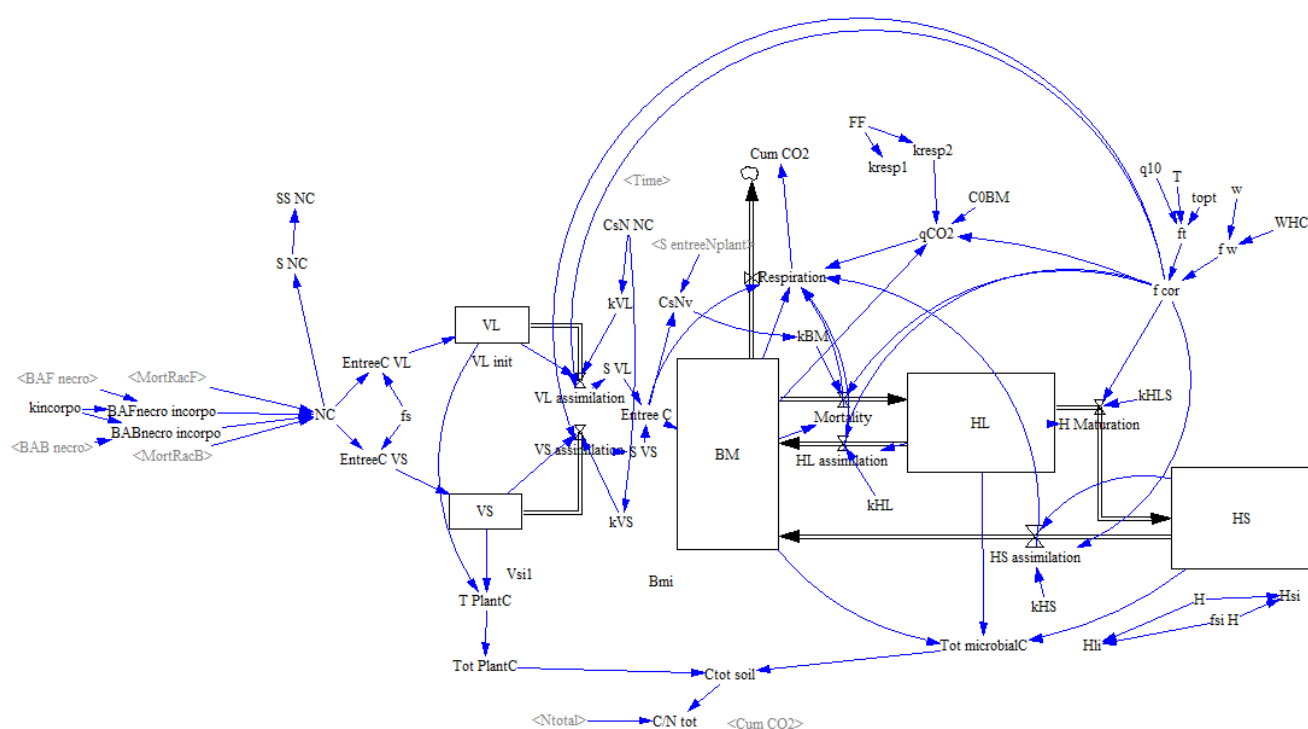
BAB is C aerial biomass of wheat, BRB is C root biomass of wheat, GrainsB is C grain biomass of wheat, MortRacB is daily root mortality of root wheat, BAB necro is C from wheat litter brought both by natural mortality and human intervention,

Culture, semis and moisson are agronomic variables: culture = 1, 2, or 3 for wheat, fababeau, and wheat-fababeau, respectively, semis and moisson = 0 for all days except = 1 for day of day of seedling or harvest respectively, f cor is the correction factor of temperature and moisture assumed to be the same for plant and microorganisms in Annex 4b (see eq. 1, 2, and 3), FacComp is a factor for plant competition.



#### Annex 4b: MOMOS-C as programmed on VENSIM for the four plant sources of carbon

BM is microbial biomass, HL and HS are labile and stable humus, respectively, NC are aboveground and belowground materials from each plant, VL and VS are labile and stable material of each NC, fs is stable fraction of each added material calculated by TAO (see above eq. 9), CsN NC is C:N ratio of each NC material, CsNv is C:N ratio of total plant material entering the soil each day, is FF 0-20  $\mu\text{m}$  mass fraction in soil, qCO<sub>2</sub> is respiratory quotient of microbial biomass, see annex5 for meaning of other parameters.



**Annex 5:** Parameter meanings and values of the MOMOS-C model obtained from previous  $^{14}\text{C}$  calibration and validation experiments (Pansu et al., 2004; 2010) and preserved in this study:

T is daily temperature,  $\theta$  is daily soil moisture in the 0-30 cm layer simulated by SAHEL model, see above equations 2, and 3 for calculation of  $f(T)f(\theta)$ ,  $\eta_{\text{NC}}$  is C:N ratio of input materials (shoots and roots of each plant for  $k_{\text{VL}}$  and  $k_{\text{VS}}$  calculations, mean predicted C:N ratio of whole NC inputs for  $k_{\text{MB}}$  calculations, see annex 4b),  $F_{0-20}$  is weight percent of 0-20  $\mu\text{m}$  fraction in soil texture.

Parameter symbol	Definition	Unit	Optimal values, link's relationships and References
fs	Stable C fraction of added NC	No dimension	TAO model, Thuriès et al., 2002 ; Kaboré et al., 2010
$k_{\text{VL}}$	Assimilation rate by MB of labile added NC	$\text{day}^{-1}$	$f(T)f(\theta) \text{MAX}(0.65-0.0019\eta_{\text{NC}}, 0.1)$ Bottner et al., 2006
$k_{\text{VS}}$	Assimilation rate by MB of stable added NC	$\text{day}^{-1}$	$f(T)f(\theta) \text{MAX}(0.0037-0.000026\eta_{\text{NC}}, 0.00005)$ Bottner et al., 2006
$k_{\text{HL}}$	Assimilation rate by MB of labile humus	$\text{day}^{-1}$	$0.05 f(T)f(\theta)$ Pansu et al., 2004
$k_{\text{HS}}$	Assimilation rate by MB of stable humus	$\text{day}^{-1}$	$0.00005 f(T)f(\theta)$ Pansu et al., 2004
$k_{\text{HLS}}$	Rate of stabilisation of HL to HS	$\text{day}^{-1}$	$0.0003 f(T)f(\theta)$ Pansu et al., 2004
$k_{\text{MB}}$	Mortality rate of MB	$\text{day}^{-1}$	$f(T)f(\theta) \text{MIN}(0.42+0.0012\eta_{\text{NC}}, 0.8)$ Bottner et al., 2006
$k_{\text{resp}}$	Respiration rate of MB	$\text{day}^{-1}$	$f(T)f(\theta) (-0.0008F_{0-20}+0.062)$ Pansu et al., 2007; 2010
$C_0^{\text{BM}}$	MB-C at steady state	$\text{g MB-}^{14}\text{C g}^{-1} \text{ added-}^{14}\text{C}$	Pansu et al., 2010